Edited by
María Luz Rodríguez Méndez

# ELECTRONIC NOSES AND TONGUES IN FOOD SCIENCE

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# Electronic Noses and Tongues in Food Science

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# Preface

Electronic noses and tongues are the products of advanced chemical and physical sciences combined with the intuitive integration of sensors, microprocessors, advanced informatics, and statistics. They include resistive, optical, electrochemical, or piezoelectrical platforms, where a variety of sensing materials (including, among many others, metal oxide semiconductors, conducting polymers, nanoparticles, phthalocyanines, or enzymes) have been immobilized using numerous different techniques.

Some of these devices are available commercially, whereas others are *home-grown* prototype devices that require commercialization. Electronic noses and tongues have been used to characterize components that contribute to sensory or compositional profiles, from ripening to harvesting and from storage of raw materials to packaging and consumption. Electronic noses and tongues are thus suitable for high-throughput analysis and quality control or for determining the nature and extent of spoilage and adulteration. These devices have also been used to ascertain the geographical origins of food and mixtures. Devices used to analyze one particular food item can theoretically be adapted for other food items or components. This does not just mean redeploying the sensing devices but also the mode of statistical analysis. This includes supervised and unsupervised tools such as principal component analysis (PCA), linear discriminant analysis (LDA), partial least squares (PLS), and artificial neural networks (ANN). In other words, there is cross-transference of chemistry, physics, concepts, techniques, findings, and approaches from one food to another. However, finding all this information in a coherent and comprehensive text has been a problem because, until now, no publication has attempted to marshal together all the relevant information on these important devices in relation to food science. This is addressed in “Electronic Noses and Tongues in Food Science.”

Its unique feature is the three parts dedicated to the electronic nose, the electronic tongue, and the combined systems of electronic nose and tongue. Part I covers a description of electronic nose systems and their applications to the analysis of the volatile composition of different foods and beverages. Part II focuses on the electronic tongue, which has become increasingly important over recent years because it can analyze complex liquids, such as wines or milk, by direct immersion in the samples and not restricted to the headspace. Part III covers newer developments combining both the electronic nose and tongue. Each part presents the main applications in the food industry. Not only classical applications in the fields of meat, wine, dairy products, or beers are presented but also other lesser-known applications, such as the detection of gliadins or the assessment of the phenolic content in foods.

This book is designed for food scientists, technologists, and food-industry workers, as well as research scientists. Contributions are from leading national and international experts, including those from world-renowned institutions. Readers can dip into the book for reference purposes, read any chapter as a standalone treatise, or read it from cover to cover if food analysis is an integral part of their day-to-day job.

I must conclude by thanking all those who have contributed to this book, each a recognized expert in their field. I also wish to thank Elsevier Publishing for all the guidance on pulling together such an eclectic book. Finally, many thanks to my colleagues and PhD students for their help and support!

**Professor María Luz Rodríguez Méndez**

## Chapter 1

# Electronic Noses and Tongues in the Food Industry

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## 1.1 INTRODUCTION

The analysis of food flavors is complicated because of several factors. The flavor active compounds are found in very low concentrations, ranging from a few hundred parts per million for strongly flavored food products to less than 10 ppm for weakly flavored foods. In development of new products, manufacturing quality control, shelf-life control, monitoring degradation during transportation and monitoring of highly perishable foods, such as fish, the use of analytical instruments are important. Monitoring of food products in terms of quality and control of production processes, such as mixing, heating, drying, cooking, baking, extruding, fermenting, and so forth are normally performed using physicochemical measurements, that is, pH-value, color, concentration of given chemicals or biomolecules generally determined by spectroscopy [Fourier transform infrared (FTIR), near infrared (NIR), ultraviolet–visible (UV–Vis), etc.]. This is due to the lack of reliable odor and taste-assessing instruments and the practical problems associated with using sensory panels for continuous monitoring of aroma or flavor. Headspace gas chromatography (HGC) and two-dimensional gas chromatography using different types of detectors, for example, mass spectrometry coupled to olfactometry (Wardencki et al., 2009) can give detailed chemical composition data as well as the information on organoleptic qualities of individual volatile compounds present in the mixture (Cordero et al., 2015). These types of analyses can be very useful in developing new products, or for detection of components that may degrade organoleptic quality, but are very time consuming and expensive to run. While these techniques are useful for analyzing volatile components for compounds involved in taste that are dispersed in aqueous medium, often in complex matrices, it become much more difficult—high-pressure liquid chromatography coupled with electro-spray mass spectrometry can give some idea of the chemical composition, but this needs to be augmented with other types of measurements for different ions, ionic strength, pH, viscosity, and so on, that give an overall impression of the food sample.

Despite advances in instrumental analysis, the flavor sensations perceived by humans can be measured only by sensory tests (Noble, 2006; Simon et al., 2008; Simons and Noble, 2003). Three types of sensory testing are commonly used, each with a different goal and using participants selected using different criteria. These primary kinds of sensory tests focus on the existence of overall differences among products (discrimination tests), specification of attributes (descriptive analysis), and measuring consumer likes and dislikes (affective or hedonic testing). Correct application of sensory technique involves correct matching of a method to the objective of the tests, and this requires good communication between sensory specialists and end users of the test results (Lawless, 2013).

The aroma components of the majority of food products consist of complex mixtures of chemicals consisting of several hundred or thousands of volatile compounds. These, together with taste components perceived as salty, sour, sweet, bitter, umami, and others combine to give to a food its characteristic flavor. Volatiles and flavor compounds can originate at every production stage from all food components. They are present in the raw materials and they can be generated during the food processing as well as during food storage (Plutowska and Wardencki, 2007).

Important flavor components that contribute to the perceived organoleptic qualities of foods include aldehydes, alcohols, ketones, acids, esters, lactones, phenols, terpenoids, sulfur-containing compounds, pyrazines, and amines. Off-odors and flavors can be generated by bacterial action on food substrates, molds, and fungi, and biochemical reactions (enzymatic reactions as well as chemical reactions).

We generally think about flavor as one of the three main sensory properties when choosing a particular food; appearance and texture are the other two properties. For humans

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the perception of flavor consists of the sensory combination and integration of odors, tastes, oral irritation, thermal sensations, and mouth feel that originate from a particular food (Breslin, 2001; Breslin and Spector, 2008). When we describe the aroma of foods, we often use the term "taste" to indicate sensations that usually are quite complex and include to a large extent smell sensations. When we introduce food into our mouth, taste receptors located on the surface of our tongue are stimulated and send signals to the brain. However, at the same time, especially as a result of mastication, many volatile components are also released, reaching the olfactory mucosa through an opening situated on the upper wall of the palate. This combination of sensations is what makes up "flavor." The general categorization of flavor can be the broad sensations perceived when consuming different foods. From a biological point of view the flavor sensation can be defined as the perception originated after a substance has been taken into the mouth and it is a specific characteristic of the substance being perceived (Labows and Cagan, 1993; Lawless, 1991). The multisensory integration that makes up our perception of flavor is extremely complex and continues to be a fascinating field of study for psychologists and biologists (Auvray and Spence, 2008; Spence, 2015).

The two main sense organs by which flavor is perceived are the nose and the mouth. Flavor comes from three different sensations: taste, trigeminal (those sensations perceived by a human as astringency, pungency, and cooling) sensations, and aroma. Trigeminal and taste sensations are realized with the presence of food in the mouth. The aroma sensation occurs when the molecules of the aromatic compound are detected at the olfactory receptors reaching this site by the nasal or oral passageways. Assessing food quality is complex, because this translates to assessing appearance, color, organoleptic qualities, and for taste—mouth feel and so forth that results in determining "liking" or "disliking."

This assessment is subjective, requiring human noses and tongues; typically a trained human panel is required. The many different possible flavors are due to interactions of chemical compounds with taste, trigeminal sensations, or aroma receptors. The characteristic taste of a food is normally related to a single class of compounds. But, an odor is usually elicited by a combination of volatile compounds, each of which imparts its own smells. Differences in characteristics of certain aromas can be equated to the varying proportions of these volatiles. However, some substances contain trace amounts of a few volatile compounds that possess the characteristic essence of the odor. These are called character-impact compounds. One must also realize that the chemicals of a single compound class can induce many diverse flavors, especially as their concentrations vary.

Sensory evaluation comprises a set of techniques for accurate measurement of human responses to foods and minimizes the potentially biasing effects of brand identity and other information influences on consumer perception. As such, it attempts to isolate the sensory properties of the foods themselves and provides important and useful information to product developers and food scientists about the sensory characteristics of their products. Sensory evaluation has been defined as a scientific method used to evoke, measure, analyze, and interpret those responses to products as perceived through the senses of sight, smell, touch, taste, and hearing.

### 1.1.1 Discrimination Tests

Discrimination tests are used when the sensory specialist wants to determine whether two samples are perceptibility different. It is possible for two samples to be chemically different in formulation, but for human beings not to perceive this difference. Product developers exploit this possibility when they reformulate a product by using different ingredients while simultaneously not wanting the consumer to detect a difference. If the difference between the samples is very large and thus obvious, discrimination tests are not useful. Therefore, discrimination testing is most useful when the differences between the samples are slight. Discrimination tests are usually performed when there are only two samples. There are a number of different tests available, including triangle tests, *n*-alternative forced choice tests, tetrad tests, and polygonal and polyhedral tests (O'Mahony, 2013).

### 1.1.2 Descriptive Analysis

Descriptive sensory analysis is a highly sophisticated technique that allows the sensory scientists to obtain complete sensory descriptions of products. They help identifying underlying ingredient and process variables, and/or determine which sensory attributes are important to acceptance. Usually, descriptive techniques produce objective descriptions of products in terms of the perceived sensory attributes. Depending on the specific technique used, the description can be more or less objective, as well as qualitative or quantitative. Most descriptive methods can be used to define sensory instrumental relationships.

### 1.1.3 Flavor Profile

Flavor profiling is a consensus technique. The vocabulary used to describe the product and the product evaluation itself is achieved by reaching agreement among the panel members. The flavor profile considers the overall flavor and the flavor noted and estimates intensity and amplitude (overall impression) of these descriptors. The technique provides a tabulation of the perceived flavors, their intensities, their order of perception, their aftertastes, and their amplitude. If the panelists are trained appropriately, this tabulation is reproducible. Using standardized techniques of preparation, presentation, and evaluation, a panel consisting of four to six judges are trained to precisely define flavors of a product within a specific food category. The food samples are tasted and all perceived notes are recorded for aroma, flavor, mouthfeel, and aftertaste. After this exposure, the panelists review and refine the descriptors used.

At the completion of the training phase, the panelists have defined a frame of reference for expressing the intensities of the descriptors used. The samples are then served to the panelists in the same form that they would be served to the consumer and the intensities of the perceived flavor notes are rated typically on a defined scale. A consensus of scores is obtained by discussion and reevaluation of the products by the panelists.

It can be seen that sensory evaluation of foods is very complex and subject to high variability, and instrumental means of carrying out some of these evaluations would be most useful to the food industry. The remarkable capabilities of the nose and tongue in detection, recognition, and discrimination of complex mixtures of chemicals, together with rapid advances in understanding how these systems operate has stimulated the imagination and interest of many researchers and commercial organizations for the development of electronic analogues of the biological systems. This chapter focuses on "electronic noses and tongues" as applied to the food industry. The responses of individual odor sensors or taste sensors combined into an array, by which each sensor possesses slightly different response selectivity and sensitivity toward the sample odors or tastants, when combined by suitable mathematical methods, can provide information to discriminate between many sample odors or tastants and when combined together—flavors. Arrays of gas and odor sensors, made using different technologies have become known as "electronic noses" and consist of three elements: a sensor array which is exposed to the volatiles; conversion of the sensor signals to a readable format; and software analysis of the data to produce characteristic outputs related to the odor encountered. The output from the sensor array may be interpreted via a variety of methods—principal component analysis, discriminant function analysis, cluster analysis, and artificial neural networks—to enable discrimination between samples. A similar approach is adopted with "electronic tongues," by which the sensors in this case detect chemical species in solution.

## 1.2 BIOMIMETIC SYSTEMS

Attempts to mimic the "chemical senses" using artificial systems are still in the process of development and have been evolving over the last few decades. One of the pioneers in thinking about the concepts, Dravnieks (1968) envisaged an instrument that would inspect samples of odorous air and report the intensity and quality of an odor without the intervention of a human nose. The ideas of a combinatorial approach utilizing arrays of broad specificity sensors outlined by Persaud and Dodd (1982) have formed the basis of many types of "electronic noses" and "electronic tongues" developed since then. The concepts are based broadly on our understanding of biological odor and taste transduction mechanisms where large numbers of different odor receptor types or taste receptors respond to the same chemical stimulus but with different affinities. The result is a complex pattern of neural responses that the brain associates with previously learned stimuli in order to identify or discriminate between these chemical stimuli. In order to understand these concepts, it is worth spending some time understanding some of these biological mechanisms.

### 1.2.1 Biology of Smell and Taste

The nose is capable of detecting a large repertoire of molecules—the latest estimates indicate that these may number over 1 trillion odors (Bushdid et al., 2014). It resembles somewhat the concepts inherent in the immune system where it is not possible predict what molecules may be encountered at any time. These molecular families include aliphatic and aromatic molecules with varied carbon backbones and diverse functional groups, including aldehydes, esters, ketones, alcohols, alkenes, carboxylic acids, amines, imines, thiols, halides, nitriles, sulfides, and ethers. With rapid advances in molecular biology, we now have a good understanding of the transduction processes in the nose and signal-processing pathways in the brain that are responsible for detection, discrimination, and recognition of odors (Firestein, 2001).

About 6 to 10 million olfactory sensory neurons (OSN) are found in the nasal cavity of mammals. Each of these cells is a bipolar neuron where the apical end ends in a knob from which protrude a number of cilia that lie in a thin layer of mucus on the surface of the olfactory epithelium (Fig. 1.1). These cilia contain the sensory transduction mechanisms constituting receptor proteins and an enzymatic cascade that transforms the energy of an odorant molecule binding to the receptor into a neural signal. There is great degree of similarity between receptors, but there is a region of hypervariability that is associated with the ligand-binding pockets of these proteins. This accounts for the large and diverse range of molecules that can be detected by these receptors (Firestein, 2001; Mombaerts, 1996, 1999).

The opening of ion channels in the cell membranes when an odorant molecule interacts with the receptors causes a change in electrical potential in the cell membrane (Chiu et al., 1997), leading to generation of action potentials that are propagated to the olfactory bulb, a structure in the forebrain devoted to the processing of olfactory signals. Despite the huge number of olfactory neurons, there are only 300–400 different types of receptors in humans. Olfactory neurons expressing a particular receptor type converge to distinct regions in the olfactory bulb called glomeruli. The

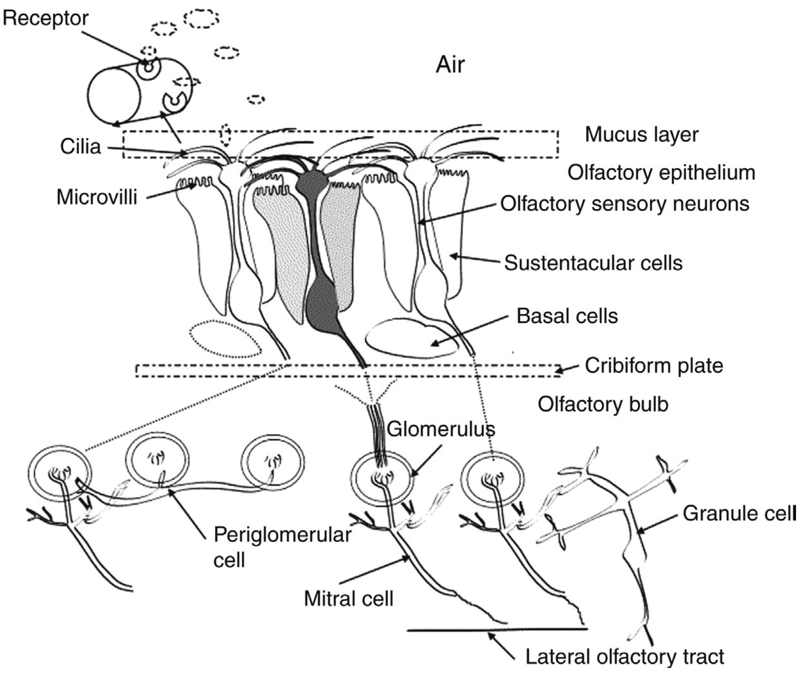

**FIGURE 1.1 The olfactory epithelium consists of OSNs, which are bipolar neurons, sustentacular cells that are glial cells with microvilli, and basal cells that are stem cells, from which new OSNs are generated.** Each OSN expresses only one of the ~1000 olfactory receptor genes and the axons from all cells expressing that particular receptor converge onto one or a few glomeruli in the olfactory bulb. The glomeruli contain the incoming axons of OSNs and the apical dendrites of mitral cells that are the output layer of cells. Mitral axons leaving the OB project to higher brain structures including the piriform cortex, hippocampus, and amygdala.

outputs from this region are specialized cells called mitral (M/T) cells that propagate a highly reduced number of signals to higher parts of the brain—many thousand olfactory neurons converge onto the dendrites of between 5 and 25 mitral cells in each glomerulus. Each ORN type projects into one or two glomeruli and a single M/T cell receives input from just one type of ORN, expressing the same type of receptor, and sends its axon to the olfactory cortex. Therefore, the first representation of the odor is transduced at the glomerular level, to produce a second spatial and very ordered pattern, representing its molecular features and distributed among a much smaller number of output cells (chemotopic coding) (Mombaerts, 1999, 2006; Mori et al., 2006; Mori and Sakano, 2011). A combinatorial strategy is adopted by the biological system (Gupta et al., 2015; Korsching, 2001; Manzini et al., 2014). Most odor molecules are recognized by more than one receptor and most receptors recognize several odors that may be related by some properties such as the size, shape, and charges associated with functional groups on the molecule. A particular odor molecule may also consist of a number of these "epitopes" or "determinants" that possess some of these features. Thus the recognition of an odorant molecule depends on which receptors are activated and to what extent.

While taste sensations have been described for many centuries, it is only comparatively recently that the fundamental mechanisms underlying taste transduction were uncovered (Kinnamon, 2012; Simon et al., 2008; Teeter and Brand, 1987). On the tongue are found structures called papillae that contain taste buds (Fig. 1.2). These papillae have different shapes: fungiform found mainly on the anterior of the tongue, foliate along the sides, and circumvallate at the back of the tongue. Within each taste bud are found approximately 50–100 cells that contain the transduction elements associated with taste. Taste receptor cells are not neurons, but interrelate with projection neurons that make up the taste nerves. In mammals, the taste buds, depending upon their location, are innervated by one of several paired cranial nerves: the chorda tympani (anterior tongue) and greater superficial petrosal (soft palate) branches of the facial nerve (CN VII), the glossopharyngeal (CN IX) nerve (posterior tongue), and the vagus (CN X) nerve (root of tongue and esophagus). The maxillary and mandibular branches of the trigeminal nerve (CN V) mediate touch, pain, temperature, and other somatosensory sensations throughout the nasal and oral epithelia—that is, coolness and fizziness. The taste fibers integrate information from taste buds and relay this information to the nucleus of the solitary tract of the brainstem. The primary taste cortex contains neurons that respond best to sweet, sour, bitter, salty, and umami-tasting agents, as well as neurons responsive to touch and smell. It has direct reciprocal connections with the frontal temporal, parietal, entorhinal, and orbitofrontal cortexes and other cortexes. The entorhinal and orbitofrontal cortex functions

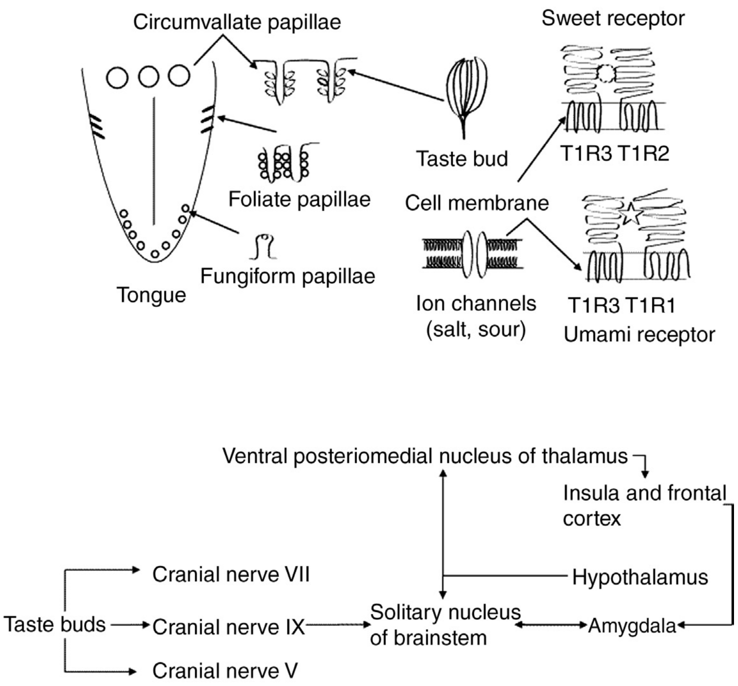

**FIGURE 1.2 The tongue showing fungiform, foliate, and circumvallate papillae.** These contain taste buds containing a number of sensory cells; membrane-bound receptors—sweet taste and umami are transduced by heterodimers of T1R2 and T1R3 subunits, and T1R1 and T1R3 subunits, respectively, while bitter taste is transduced by T2R receptors (not shown). Sour taste involves activation of a type of transient receptor potential channel (TRP), while salt taste is transduced via epithelial sodium ion channels in the cell membrane. The signals from taste buds project to the solitary nucleus of the brainstem, and then is processed by higher regions of the brain.

to interpret tastes and includes memory and cognition. The insular cortex is associated with conditioned taste aversions.

The cell membranes of the taste bud cells contain a variety of ion channels that are involved with signal transduction. Salt taste is transduced via an epithelial sodium ion channel (ENaC) that is ubiquitously expressed and functional in the anterior part of the tongue. Sour is the taste of acid, that is, protons ($H^+$). This taste is complex and is transduced through transient receptor potential (TRP) ion channels, but there may at least three possible receptor mechanisms: $H^+$ blocks $K^+$ ion channels, $H^+$ ions go through ENaC channels, or $H^+$ ions go through a proton channel.

Sweet and umami receptors are heteromeric receptors made up of a combination of different subunits, coded for by a small gene family—T1R. Sweetness receptors are a combination of two types of receptor subunits (T1R2 + T1R3) and umami receptors a combination of another set of receptor subunits (T1R1 + T1R3) (Doty, 2012). The bitter receptors consist of taste-2 receptors (T2Rs). Fifty to eighty members are expressed in small subset of all taste papillae. T2Rs are membrane bound proteins that are bitterness receptors (Meyerhof et al., 2011). We still poorly understand taste, and other types of receptors that are under investigation include those involved in the taste of fats (DiPatrizio, 2014; Passilly-Degrace et al., 2014). It is clear however that like olfactory receptors, taste receptors are capable of interacting with many types of substances with different affinities, so exhibit broad selectivity in terms of ligands that can be detected.

## 1.3 ELECTRONIC NOSES AND TONGUES

### 1.3.1 Electronic Noses

So-called "electronic noses" comprise a vapor sampling system, an array of chemical sensors, and a method of signal processing that leads to classification of the responses of the chemical sensors. The sensors in an electronic nose are desired to have a broad selectivity rather than being specific to one type of volatile chemical. The human nose can identify many odors that may contain hundreds of individual chemical components and, therefore, the sensors for an electronic nose should be generalized at the molecular level. The desired properties for sensors are high sensitivity, rapid response, good reproducibility, and reversibility to large numbers of chemicals. It is also better for an electronic nose to be small in size and flexible, able to adapt to being exposed to many types of environments, and operate at ambient temperatures. The working principle of these instruments is based on the employment of an array of different nonselective chemical sensors. Each sensor in the array is able to detect a range of different odors, not just one, with a sensitivity that is different from the sensitivity shown by the other elements of the array. Such features make the

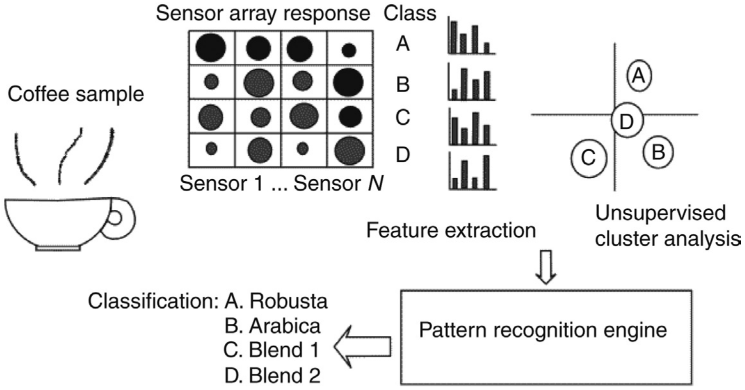

**FIGURE 1.3** Electronic nose concepts: sampling, sensor array, signal processing, pattern recognition.

response of the electronic nose dependent on the whole range of chemical information contained in an odor. This is something similar to what occurs in biological olfaction.

Usually, gas sensors are categorized based on their transduction principle and sensing material. Many different types of sensor technologies have been so far deployed (Gutierrez and Horrillo, 2014; Vergara and Llobet, 2011; Wilson and Baietto, 2009)—these include metal oxide sensors, conducting polymers, optical sensors, piezoelectric sensors, electrochemical sensors, field effect transistors (Feng et al., 2014), and more recently olfactory biosensors containing either olfactory receptors (Ault and Broach, 2006; Du et al., 2014) or odorant-binding proteins (Di Pietrantonio et al., 2013; Pelosi et al., 2014; Persaud, 2012) have been utilized. The interaction with an analyte induces a physical and/or chemical change in the chemical sensing layer, which produces a signal. The nature of the transduction processes utilized by these sensors may include electrical measurements, including changes in current, voltage, resistance or impedance, electrical fields, and oscillation frequency. Other transducers involve measurements of mass changes, temperature changes or heat generation. Optical sensors measure the modulation of light properties or characteristics such as changes in light absorbance, polarization, fluorescence, and other optical properties. These have been extensively reviewed in other publications (Di Natale et al., 2005, 2009; Walt and Sternfeld, 2006; Walt, 2010) and will not be described in detail here. The choice of sensor is often application dependent and are based on response and recovery times, sensitivities, detection range, operating limitations, physical size, robustness to being poisoned, power consumption, and other factors. However, very few types of chemical sensor available respond specifically to a single chemical—they tend to show cross-sensitivity to a variety of chemicals, but the range of selectivity can be tailored so that each type of sensor responds to a different range of chemicals. This inherently poor selectivity can be utilized to advantage when sensors of different type are combined into an array. The cross-reactive sensor array is composed of different sensors chosen to respond to a wide range of chemical classes and discriminate mixtures of volatile compounds that make up an odor. The outputs from individual sensors are processed in such a way in order to produce a distinct response pattern reflecting the relative responses of all the sensors in the array to a given chemical stimulus. Identification and classification of an analyte mixture is accomplished through comparison of this electronic fingerprint of sensor responses against previously learned patterns (Fig. 1.3). Unlike conventional analytical instruments, there is no need for separation of a complex mixture into individual components and this holistic approach resembles the process found in biological olfaction. Importantly, these devices give information that allow comparison or discrimination of odors without necessarily having to do a chemical analysis of individual components in the mixture. There has also been a blurring of instrument technologies where “electronic nose” concepts are combined with traditional analytical instruments; for example, a chromatogram may be treated as a set of virtual sensors, or direct injection of a mixture of substances into a mass spectrometer without prior separation will produce a complex spectrum that can be processed as a “fingerprint” to describe that mixture (Crespo et al., 2012; Moon et al., 2014). Taking this to the opposite extreme, modulation of a single sensor can produce a virtual sensor array—for example, modulating the operational temperature of a single metal oxide sensor can process a complex response that can be deconvoluted (Amini et al., 2013; Herrero-Carron et al., 2015). On the other hand, large arrays of sensors consisting of up to 4096 sensors have been described and these start to resemble more realistically the olfactory receptor system (Beccherelli et al., 2010; Bernabei et al., 2012).

While a number of companies were formed to exploit “electronic nose” technologies over the last few decades, the majority of these have failed. One reason is “overselling” of what such devices can actually do. In fact they are not analytical instruments and neither are they even close to the functionality of the biological nose (Boeker, 2014).

Another problem has been that of calibration of such instruments, and that of "drift" over time. The latter is associated with limitations of the sensor technologies utilized—changes in responses over time due to aging, poisoning, or environmental factors, together with changes in the chemical analytes that are being assessed. For example, a typical food product will change organoleptic characteristics over time due to aging, decomposition, or other factors, and different batches of a product may have large differences in odor or flavor. Nonetheless, there have been real successes in the use of these technologies in the food industry that shall be described later.

The performance of today's artificial systems is far from that found in biological olfaction. The better performance of the latter is due to the great number of receptor neurons and to the unique architecture of the olfactory pathway, where three main elements, the olfactory epithelium, the olfactory bulb and the olfactory cortex, are completely integrated. In the artificial olfactory devices, similarly to the brain, the signals coming from the sensors are processed in order to classify odors. However, this process is carried out using mathematical tools. An initial sensor signal preprocessing is often performed. Different preprocessing metrics can be used with different aims. For example, the time dependence of the signal can be removed in order to reduce the amount of data that needs to be handled during the phase of pattern recognition. Linearization of the sensor response can be performed when the intensity of the odors is important, as the sensor response is often nonlinear with increasing concentration of analyte. Data reduction techniques, such as principal component analysis (PCA), imitating the massive convergence of the ORNs to the glomeruli, can also be applied to the whole sensor array response, in order to reduce the data dimensionality (Pearce, 1997a,b).

The data resulting from this first processing step, or coming directly from the sensors without any pretreatment, are subjected to pattern recognition (PARC) analysis to obtain discrimination and/or classification. During this phase, the sensor patterns are compared to known odor patterns, which have been stored in a knowledge base during a previous training stage of the device, and, using any of the many classification techniques available, identification of the stimulus is carried out. Existing sensor arrays implement the broad and overlapping sensitivity feature exhibited by biological receptors, but it has not yet been possible to achieve the same dimensionality and redundancy level available in the olfactory epithelium. Current chemical sensor arrays, either homogeneous or heterogeneous, have few sensing elements when compared to natural noses.

## 1.3.2 Sampling

Apart from sensing aspects associated with "electronic noses," the sampling aspects are often neglected. In order to get consistent results from such devices—especially when measuring a complex matrix such as food—much care needs to be taken and in fact one complaint often heard is the amount of work required for method development for a particular application. The techniques that are used are those that have been well developed over the years for gas chromatography and have been adapted for "electronic nose" detectors (Feng et al., 2014; Pillonel et al., 2002).

The simplest technique consists of placing the sample into a vial that is then closed and allowing equilibrium to be reached between the matrix and the vapor phase. This "static headspace" of the vial then constitutes the sample that is introduced into the "electronic nose" system. The sample temperature, equilibration time, vial size, and sample quantity are parameters that have to be optimized for a particular application and often this can be automated.

Instead of a generating a static headspace, the sample may be placed into a vial and in this case the vial is purged with a continuously flowing gas stream. This in effect strips volatile components from the matrix of the sample as there is a displacement of the equilibrium between the sample matrix and the headspace. This "dynamic headspace" has been used to good effect in many applications. The flow rate of the gas and the sample temperature needs to be optimized for a particular application.

Often the concentrations of volatile components from a sample are very low, and the dynamic headspace method may be combined with a preconcentration method such as "purge and trap" where the vapor is trapped on an adsorbent such as activated carbon, polymers, or inorganic matrices. After sampling the dynamic headspace over a period of time, the trap is heated to a high temperature in order to discharge the trapped volatiles as a bolus into the electronic nose. The flow rate of the gas, the purge rate, and the desorption temperature are parameters that need to be adjusted for a given application (Chai et al., 2008; Chen et al., 2015; del Nogal Sanchez et al., 2014; Feng et al., 2014; Lopez-Feria et al., 2008; Pillonel et al., 2002; Plutowska et al., 2011).

Solid-phase microextraction (SPME) introduced by Pawliszyn et al. (2012) and Pawliszyn (2012) and stir-bar adsorption (SBE) are convenient methods that are used to preconcentrate a static headspace (Baltussen et al., 2002; David and Sandra, 2007). In the former a silica fiber coated with an organic polymer is introduced into the headspace and this sorbs volatile components from the headspace and entraps them. A range of sorbent coatings are available that range from hydrophilic to hydrophobic so some selectivity in what is extracted from the headspace is under control of the user and can be adapted to a particular application. SBE uses a similar principle but contains a much larger adsorbent surface allowing much greater quantities of volatiles to be entrapped. Just like the purge-and-trap method, the SPME or SBE is heated to a high temperature to desorb volatiles into the electronic nose detector. A variation of

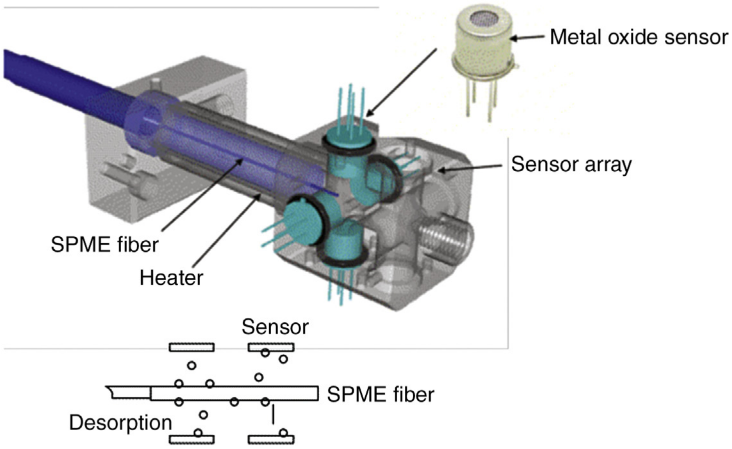

**FIGURE 1.4** **Solid-phase microextraction of a headspace and desorption process of an SPME fiber on to an array of sensors.**

this technique uses a needle where the inner surface is coated with an adsorbent and the vapor is drawn through the needle to be entrapped. Fig. 1.4 shows the introduction of an SPME fiber into an array of metal oxide sensors for electronic nose applications.

Apart from these common sampling techniques, specialized systems exist that are dependent on the detectors that utilized in the "electronic nose"; for example, for direct inlet mass spectrometry, a membrane is inserted between the sample and the inlet of the mass spectrometer, allowing controlled introduction of sample volatiles directly into the ion source. Sampling techniques are reviewed by Rubiolo et al. (2010).

### 1.3.3 Electronic Tongues

In principle, "electronic tongues" function in a similar way to the "electronic nose" (Fig. 1.5). A sensor array produces signals that are not necessarily specific for any particular chemical species. A pattern of signals is generated, that can be correlated to certain features or qualities of the sample. In this case the sensors operate in an aqueous environment and they have different cross-sensitivities to various chemical species. Just like the "electronic nose," an appropriate method of multivariate analysis or pattern recognition is used to process the signals from the array in order to discriminate or classify different samples. As with "electronic noses," a wide variety of sensor technologies may be employed. These include conventional ion selective electrodes, chalcogenide and oxide glasses, noble metals, organic polymers, biosensors, ISFETS, optical sensors, mass sensors, impedimetric sensors, and others (Baldwin et al., 2011; Legin et al., 2004, 2005; Vlasov et al., 2008, 2010; Winquist et al., 2004). The most common sensors used are potentiometric in nature, producing an electrical potential described by the Nernst equation, Eq. (1.1),

$$E = E^0 - \frac{RT}{nF}\ln Q \tag{1.1}$$

where, $E$ represents the observed cell potential at zero current, $E^0$ is the standard cell potential, $R$ the universal gas constant, $T$ the absolute temperature in Kelvin, $n$ is the charge number of the electrode reaction, $F$ is the Faraday constant, and $Q$ is the ratio of ion concentration at the anode to ion concentration at the cathode. They include ion selective electrodes. The selectivity of these electrodes is described by the Nikolsky–Eisenmann equation, Eq. (1.2),

$$E = E^0 + \frac{RT}{z_i F}\ln\left[a_i + \sum_j (k_{ij}a_j)^{z_i/z_j}\right] \tag{1.2}$$

where $a_i$ and $a_j$ are the activity of the primary ion and interfering ion, respectively; $k$ is the selectivity coefficient; $E^0$ is the sum of the standard potential of the electrode and the junction potential; $E$ is the potential difference for the electrochemical cell composed of the ion selective and reference electrode; and $z_i$ and $z_j$ are charge numbers of the primary and interfering ion, respectively.

Because the matrix containing the analytes of interest may be very complex, the biggest problem encountered with "electronic tongues" is that of electrode fouling. Potentiometric measurements are temperature dependent, and the signals may be influenced by solution changes, as well as adsorption of solution components on membrane surfaces, that can affect the nature of the charge transfer. Hence, care needs to be taken to control the temperature of the measurement, washing of the electrodes with solvents to minimize the effects of adsorption, using antifouling membranes. However, potentiometric measurements are widely used because of their simplicity. Toko and coworkers (Tahara et al., 2013; Tahara and Toko, 2013; Toko, 2004, 2014;

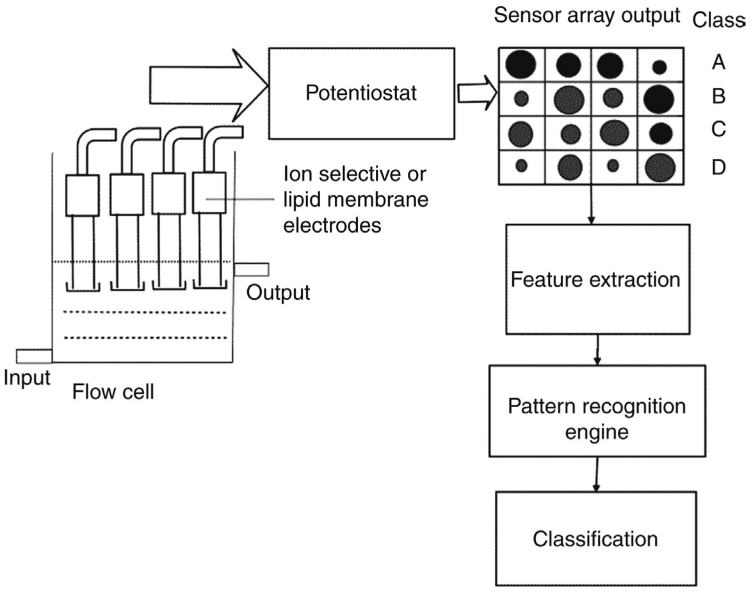

**FIGURE 1.5** Electronic tongue concepts: sampling, sensor array, signal processing, pattern recognition.

Toko et al., 2014) developed a taste sensor array based on electrodes using a lipid/polymer membrane for the transducer. The composition of the membrane is designed considering the charges on the membrane surface and hydrophobicity on the basis of physicochemical properties of substances with each basic taste, and as a result the system correlates well with basic taste perception. However, it must be realized that these systems in no way resemble the biological tongue. Unlike the case with potentiometric sensors, voltammetric measurements are performed when equilibrium is not reached, and the signal obtained is the current–potential relationship. These methods are particularly useful when trying to obtain measurements from samples that contain redox species.

Just like with electronic noses, attention needs to be paid to sampling—often static measurements are carried out, where the electrodes are inserted into a medium and an equilibrium is allowed to be established. On the other hand, dynamic flow of analyte medium past the electrodes may also give useful information.

## 1.4 PATTERN RECOGNITION

For both "electronic noses" and "tongues," pattern recognition algorithms play a large role. The notable advantage is the ability to characterize complex mixtures without the need to identify and quantify individual components, without the need of highly selective sensors. There are two main approaches to pattern recognition: parametric and nonparametric. Parametric methods rely upon obtaining or estimating the probability density function of the parameters used to characterize the response of a system. Conversely, nonparametric methods require no assumption about the fundamental statistical distributions of data. Two types of nonparametric learning or classification methods are available: supervised and unsupervised. Supervised methods involve the learning of data based on advance knowledge of the classification, whereas unsupervised methods make no prior assumption about the data.

Preprocessing of data is often necessary before pattern recognition algorithms can be applied. This may involve scaling of data, normalization, removal of redundant data, extraction of features that are important to classification, and others (Raman et al., 2011; Roeck et al., 2008). If the data are high dimensional, for example, when there are a large number of sensors in an array, or if virtual sensors are being utilized from a mass spectrometer or gas chromatograph, then principal components analysis is a powerful tool for data reduction. It involves forming a covariance matrix between variables, and then carrying out a transformation that results in extracting eigenvectors and eigenvalues. This analysis produces principal components that are a linear combination of the original variables. Many algorithms are described in the literature that are applicable to the classification problems encountered using electronic noses and tongues—they include independent component analysis (ICA) and linear discriminant analysis (LDA), learning vector quantization (LVQ), self-organizing map (SOM), artificial neural networks of a variety of architectures (feed forward–back propagation networks, radial basis functions, support vector machines, genetic algorithms, and others). All of these methods function well, but are dependent on the stability of the sensor responses over time, and consistency in sampling. The strategy is to expose the sensor array to a range of different analytes, memorize the resulting patterns in a data base, and use this a priori knowledge to recognize unknown samples later on.

## 1.5 APPLICATIONS TO THE FOOD INDUSTRY

There are a huge number of published applications of electronic noses and tongues in the food industry. These have been extensively reviewed (Ciosek and Wroblewski, 2007; Escuder-Gilabert and Peris, 2010; Peris and Escuder-Gilabert, 2009) and it must be noted that many successes are reported in monitoring the quality of foods, beverages, and pharmaceuticals. The tasks typically carried out are detection and discrimination. They include monitoring the quality of black tea, green tea, alcoholic beverages, adulteration of olive oil, rancidity of meat, fruit ripening, and others. However, it must be also said that many of these studies are not readily repeatable, and each researcher has had to carry out extensive method development where the answers to specific questions could be determined. One example is the work carried out by Vestergaard et al. (2007), who used an ion mobility-based electronic nose system for prediction of sensory quality changes of a meat-based pizza topping during storage. They showed that by projecting two independent data sets of "known" production samples and "unknown" samples purchased from a local supermarket onto calibration models, evidence was given for the predictability of the electronic nose regarding storage time and sensory quality changes during storage. This is extremely promising, but if the production parameters change or a new recipe is introduced, then it is likely that this work of calibration would need to be repeated from the beginning.

The important trend recently has been to fuse different sensing technologies together (Gutierrez-Capitan et al., 2014; Lvova et al., 2015). The former used different microsensors, while Lvova et al. used porphyrin films as sensing materials for an electronic tongue, simultaneously measuring optical properties, electrochemical amperometric or potentiometric response to analytes. Cole et al. (2011) have combined an electronic nose and tongue together.

It is notable that there are also some extremely important findings in terms of correlation of electronic noses and tongues with human sensory perception. Haddad et al. (2010) did an interesting experiment. They set out to determine whether electronic nose measurements can be linked to olfactory perception. There is evidence that the primary perceptual axis of human olfaction is odorant pleasantness. This is reflected in part in the physicochemical structure of odorant molecules. They tested a hypothesis that an electronic nose can be tuned to the pleasantness scale, and then used it to predict the pleasantness of novel odors. They found that their system consisting of an array of 16 sensors was able to generate pleasantness ratings with greater than 80% similarity to human ratings and with above 90% accuracy in discriminating pleasant from unpleasant odors.

## 1.6 CONCLUSIONS

Electronic noses and tongues have undergone great developments over the last few decades. The availability of a large range of sensor technologies, combined with advances in microelectronics, signal processing software, chemometric and neural network pattern recognition algorithms, together with associated methods for correcting drift in sensors and systems, have increasingly made such systems more robust. Such systems have now become hybrid—fusing many types of data together to produce outputs that can now be correlated with human sensory perception. Increasingly we shall be seeing more and more applications of such systems in "online" or "at line" applications in the food industry ensuring quality and safety of such products.

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Chapter 2

# Apple Analysis and the Electronic Nose

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## 2.1 INTRODUCTION: APPLE ANALYSIS USING AN ELECTRONIC NOSE

In general, random and destructive sampling techniques are often used in apple quality evaluation. However, a nondestructive and fast test technique needs to be developed for assessing apples and other food quality. The developing electronic nose (E-nose) is a novel instrument for analyzing, distinguishing, and detecting complex flavor and most volatile compounds since 1982. In recent research, various types of E-nose have been applied in food quality control (Schaller et al., 1998), medical diagnosis (Schiffman et al., 1997), and homeland security. It has been widely applied for predicting fruit (apple, orange, peach, etc.) ripeness and shelf-life due to its nondestructive sensitivity and good repeatability. Meanwhile, E-nose offers an objective, accurate, fast, and broad evaluation.

The apple is one of the most frequently consumed commodities in our daily life. However, during the process of picking, storing, transporting, processing, and packaging, apples are easily damaged and they deteriorate. The basic requirement of a good quality apple is maintaining the appearance, fresh taste, healthy smell, and suitable maturity. The apple's aroma is a significant indicator of estimating the apple's ripeness.

Currently, there are two ways for volatile detection of the apple's flavor. The first is a trained human sensory panel named olfactometry, which has a low sensitivity. People cannot detect volatile compounds without odor. What is more, the result is not accurate enough because it will be different from person to person and from time to time. The second is detected by instruments such as a gas chromatograph and mass spectrometer (GC-MS), E-nose. Compared with traditional instruments, E-nose exhibits several advantages including fast, convenient, nondestructive evaluation of fruit quality and ripeness and low cost, easy operation. In this chapter, we briefly summarize several types of E-nose applied in nondestructive apple detection, maturity evaluation, predicting shelf life, and so on. Several E-nose commonly used by researchers in recent years are listed in Table 2.1.

Changes in the aroma of Royal Gala apples were detected using both classical headspace/GC and E-nose (Young et al., 1999). The same techniques were also used to evaluate stored apples. Various flavor volatile indicators can be determined effectively using E-nose equipped with multiple sensors that offer great advantages in selectivity and sensitivity. Yong used Fox 4000 (Alpha MOS, France) fitted with P30/1, P10/1, P10/2, P40/1, P40/2, PA3, P70/0, T50/3, PA2, T50/1, T40/1, T70/2, SY/LG, SY/G, SY-cG, SY-gW, SY-W, and SY-gCT sensors to evaluate apples. The electrical signals were measured at 1s interval output from sensors. Principal component analysis (PCA) results showed little discrimination between the first stage apples picked before the commercial harvest criteria and the second stage picked two days later. The last two stages were apparently different from the first two stages. Discriminant function analysis (DFA) results for E-nose data showed that the four groups were more diffuse after storage. According to the results, the two methods mentioned in the article by Young et al. (1999) were capable of classifying the storage apples into four different harvested groups. However, compared with the classical way, E-nose was faster and much less complex.

Model Cyranose 320 E-nose, which contains an array of 32 carbon black organic polymer composite sensors and can function at ambient air temperature, is also used to detect the apple's maturity and defects. Cyranose 320 E-nose is relatively stable when exposed to water vapor. PCA, multivariate analysis of variance (MANOVA), and discriminant analysis (DA) are used to analyze three maturity indexes—starch, puncture strength, and soluble solids—so we can effectively classify apples into immature, mature, and overmature groups. The maturity of Gala apples was detected by Cyranose 320 E-nose (Pathange et al., 2006). Based on the maturity indexes, they obtained an objective evaluation. From the results of PCA and DA, we found it is possible to categorize Gala apples into three maturity groups by using E-nose technology, but we need more training to improve E-nose's practical applicability, such as testing in warehouses and monitoring the optimal harvest date.

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**TABLE 2.1** Common E-nose Model Used in Apple Detection

| Model | No. of Sensors | Technology |
|---|---|---|
| Fox 2000, 3000, and 4000 | 6, 12, and 18 | MOS sensor |
| PEN2, PEN3 | 6 | MOS sensor |
| Cyranose 320 | 32 | Conducting polymers |
| Libranose | 8 | QCM |
| TGS | 9 | Tin-oxide gas sensor |

Two instruments, Cyranose 320 E-nose and Z-nose, were chosen to detect apple spoilage and its volatile profiles (Li et al., 2007a). Z-nose, a detecting instrument, consists of one capillary column and one surface acoustic wave (SAW) sensor. Based on the different solubility of volatile compounds and the different time to enter the SAW sensor, the mixture could be separated in the capillary column. The data was detected by both instruments to analyze the feature-level and the decision-level multisensor data fusion models with covariance matrix adaptation evolutionary strategy (CMAES). In this way, the detection and classification performance for damaged apples will be improved than when using individual instruments alone. In the feature-level fusion, the optimized algorithm CMAES was used for feature selection in the fusion process. Two real number coding methods and three feature-based fusion schemes were developed and compared. Results showed that the 48-variable coding slightly outperformed the 24-variable coding, when search quality, search efficiency, and reduced dimensionality are taken into consideration and dynamic selective fusion performed better than the other two schemes. In the decision-level fusion, the dynamic selective fusion model performed best with lower level data fusion. This research provides a new multisensor data fusion model to detect spoiled and diseased apples. With improving detection accuracy, this model will have much practical application in food storage.

Cyranose 320 E-nose and Z-nose were used to detect deterioration in cut and uncut apples (Li et al., 2007b). In this research, both E-nose and GC-MS were used. GC-MS results showed a good performance in detecting key compounds of apple aroma change between the normal apple and the apple exposed to artificially induced damage after six days. The data of E-nose and Z-nose were compressed by PCA and partial least squares (PLS); after that linear discriminant analysis (LDA) and canonical variate analysis (CVA) models were developed on the compressed data. The results found that the volatile compounds of undamaged and damaged apples would change and become different from each other. Experiment results also showed that the number of cuts were related to volatile compound emissions, but orientation of cuts were not. Both E-nose and Z-nose were capable of detecting effectively. Furthermore, according to the research, the number of sensors was successfully reduced by 82% with improving classification accuracy. On the other hand, reducing the number of sensors potentially shortens data processing time, reduces cost, and even affects accuracy.

Objective quality of "Fuji" apples was assessed by using three different sensors: a near-infrared spectrophotometer (NIR), a machine vision system (MV), and an E-nose system (Zou et al., 2010). E-nose used in this investigation consists of a pattern recognition system and a gas sensor array containing nine tin-oxide Taguchi-type gas sensors manufactured from Figaro. Each sensor generated 4 kinds of signals, and there were 36 different signals for 9 sensors. We could establish an online predicting method of the rotting stage with the help of an artificial neural network model. A decision tree that imitates the quality detection process of a three-sensor fusion for apple quality assessment was built (Fig. 2.1). E-nose can detect whether the apple quality is good or not from the obtained aroma data analyzed by artificial neural network (ANN). NIR provides the sugar content; surely, it is better to fuse three sensors. The data of multiple linear regression (MLR) showed that there was a relationship between sugar content and different NIR wavelengths. And machine vision can provide data of size, color, shape, and some other image information, to classify sweet apples. All the sensors were working at the same time. As a result, it demonstrated that a high level of three-sensor fusion technique could improve the accuracy of quality assessment of apples and classification.

The quality of postharvest apples was evaluated by means of an E-nose (Di Natale et al., 2001). This E-nose consisted of seven thickness shear mode quartz resonators (TSMR), and detected the quality characteristics of harvested apples with the frequency of 20 MHz. In this experiment, E-nose was used to detect defects caused by over-ripening (mealiness) and skin damage (cuts), but it was more sensitive to the presence of cuts than the mealiness. Data was analyzed by PLS, which was used for discriminant analysis (PLS-DA). Results showed that the increase of mealiness did not change the compounds in apple headspace but rather in their concentration. Higher concentrations due to the oxidization process in apples from skin cuts would produce different compounds and predicted the shelf life after analyzing the results.

Determining the optimal harvest date of apples by using E-nose was investigated in 2003 (Saevels et al., 2003). The

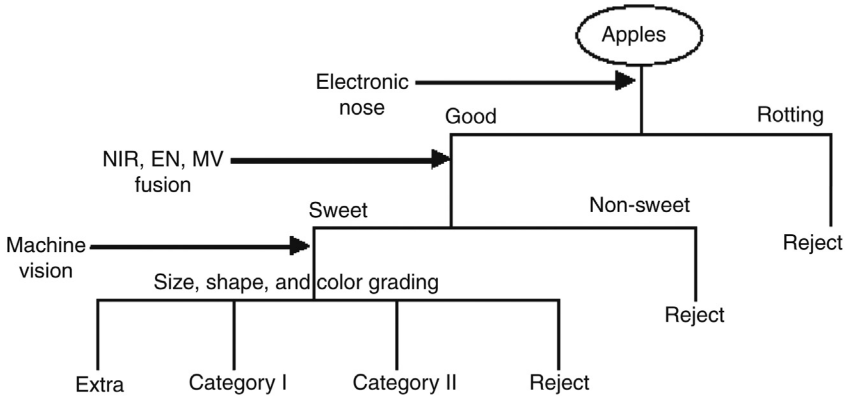

**FIGURE 2.1** **Decision tree for the assessment of the final quality of apples by three sensors.**

harvest date was based on five different quartz microbalance (QMB) sensors. However, the PCA on the date of consecutive years uncovered the presence of a year effect and the PLS was established on the date of both years, so it was difficult to build the right model on those results. Therefore, according to the Streif index, if the cross-validation correlation attained results between 0.89 and 0.92, the prediction of apple maturity would be comparably accurate. In addition, the soluble solids, firmness, and starch content could be measured on the date by using E-nose when the cross-validation correlation values were between 0.70 and 0.80. There would be a good correlation between the E-nose detection and apple maturity; the key to this technology was the practical use of E-nose. Results might predict the optimal harvest date of apple, but it was expensive and time consuming. The technique would become more practical; biological variability would be reduced because the samples could be detected in time.

In another experiment, E-nose and the mass spectrometry-based E-nose (MSE-nose) were used to evaluate apple quality during shelf life (Saevels et al., 2004). Seven QMB sensors were used in Libra nose. At the same time, the results were compared with traditional methods such as GC-MS. In this experiment, apples were stored at three different storage conditions, and the volatile profile would change every several days. Saevels found E-nose measurements showed no shelf-life or storage history effect by PCA. However, the MSE-nose and GC-MS did. Apples' volatility was similar if stored at ultra low oxygen levels and controlled atmosphere conditions, which were different from those stored under regular storage. The straight-chained esters and α-farnesene were considered to be the main factors to be determined. Because the sensors were not sensitive to some differences in straight-chained esters of apples stored under the different conditions, it was difficult to achieve the desired results by using the E-nose alone, but the GC-MS and MSE-nose could achieve better results. MSE-nose could offer the apple volatile analysis by GC-MS to predict the days of shelf life of the apples based on the PLS models.

The characteristics of Fuji apples from different harvest dates and storage conditions were detected by the GC and E-nose (Echeverría et al., 2004). The "Libranose" E-nose has seven QMB sensors with different metals, combined with chromatographic measurements to measure the volatility components, and the results showed clear distinctions between the apples under the different storage conditions. Apples were divided into four different atmospheres, a normal cold atmosphere and three controlled atmospheres. In PCA, different sensors responded to different volatility components of apples under different storage atmospheres, the date could only identify the storage periods, days of shelf life, and harvest dates but it was difficult to estimate the different cold storage atmospheres. So combining the GC and E-nose will be more useful in detecting apple storage problems.

The E-nose PEN2 was used to study the effect of natural antimicrobials to prolong the shelf life of apples (Siroli et al., 2014). This E-nose is composed of 10 temperature-moderated metal oxide sensors (MOS); different sensors are sensitive to different volatile molecules. Fresh fruits are easily infected with microbials, which lead to a loss of sensory quality. In order to increase the shelf-life quality of apples, Siroli and Patrignani tried to use natural antimicrobials to replace the traditional methods. Citron EO, hexanal, 2-(E)-hexenal citral, and carvacrol were used either alone or in combination. Compared with traditional methods, after the treatment, the apples were packaged in medium permeability bags and stored at 6°C. Then E-nose was used to determine color and texture analysis. On the basis of PCA analysis, the results showed that apples treated with the combination of those natural antimicrobials would result in good retention of many desirable qualities.

An innovative technology was used to investigate the microstructure and olfactory quality of apples through PEN3 E-nose commercially produced by Airsense Company (Airsense Analytics GmbH, Germany). It combined the Win Muster and Airsense Analytics (Airsense Analytics GmbH, Germany) to analyze the olfactory quality of

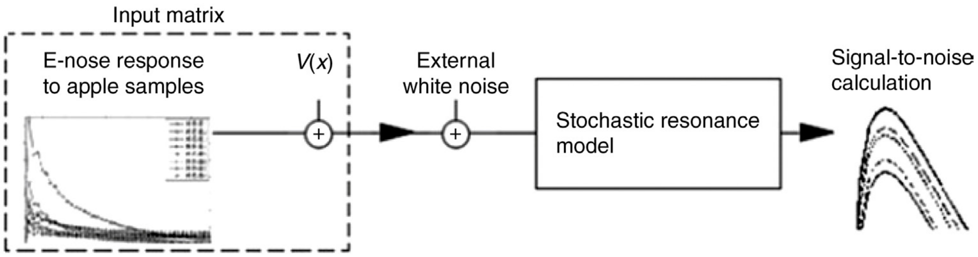

**FIGURE 2.2** **Graphical illustration of the E-nose date analysis method.**

apples (Laurienzo et al., 2013). In this experiment, Laurienzo and coworkers wanted to take advantage of natural polysaccharides to replace the classical method of dehydration of apples. An environmental scanning electron microscope (ESEM) was used to detect structural changes, and E-nose was used to detect olfactory characteristics about different dried apples. With the different biofilms, differences in microstructure of the dehydrated apples were found. The packaging with different biofilms also influenced the volatility of apples. Hence, the most intact cell walls have better retention of olfactory properties, and good methods should not damage the cell walls, and the olfactory quality of dehydrated apples should be close to those of fresh apples.

Almost all of the apple analysis methods using E-nose are usually combined with instrument analysis methods, such as GC, GC-MS, and high-performance liquid chromatography (HPLC). But these methods exhibit some disadvantages because they incur high costs and are time consuming. In our experiment, we used the home-made E-nose to investigate the apple storage time at room temperature (Hui et al., 2013). The E-nose system included three main parts: data acquisition unit, sensor array unit, and power supply unit. The sensor array consisted of eight semiconductor gas sensors.

Although the PCA method could distinguish the fresh and medium apples from overripe apples, it is hard to discriminate between fresh and medium apples. So a new method, the signal-to-noise ratio (SNR) spectrum, was calculated using stochastic resonance (SR). The prediction model of apple storage time was established by the SNR maximums (max-SNR) method. The error rate of this model was less than 10%. The equation of storage time prediction of apple could be described as:

$$\frac{dx}{dt} = -\frac{dV(x)}{dx} + MI(t) + C\xi(t)$$

The graphical illustration of SR processing is shown in Fig. 2.2.

Experimental results demonstrated that the predicting accuracy of this model is 84.62%. This method showed many advantages, including easy operation, rapid detection, a bargain price, and good repeatability. In fruit quality determination, this technology revolution would become widely popular.

## ACKNOWLEDGMENTS

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## Chapter 3

# Electronic Nose in Dairy Products

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## 3.1 INTRODUCTION

Milk is the raw material used in the manufacture of several food products. Milk varies with the animal source, its seasonal feeding, and hygiene conditions. It is a highly perishable good, and its quality must be controlled from collection to transport and storage. Milk can be consumed after several heat treatments but it can be transformed into many other products such as butter and cheese. Off-flavor odors are known by consumers as the first alarm signal linked to spoilage, and adjectives as sour and rancid are well known. Besides being used to detect quality problems, smell is also a source of pleasure, and of recognizing familiar quality products. Electronic noses can be seen as devices that help to achieve product quality certification and classification.

## 3.2 CHEESE ODOR

Most of the best cheeses are stinky. J.K. Jerome (2009) told a hilarious story about a man who sent two cheeses to his family. After a journey during which travellers tried to run away from the smell, the cheeses were delivered to his wife with the recommendation of not eating them before his return. His wife and children could not stand the smell and moved to a hotel, while the charwoman, who was not able to perceive the malodor, remained in the house. Later, the man tried to get rid of the stinky cheeses, and after a few damping locations had been tried, always with complaints from the locals, the man buried the cheeses on the beach of a seaside town, which gained quite a reputation. Visitors commented the strength of the town's air, and it became famous among lung patients. This story shows that the degree of odor perception is not the same for everybody, but also that its hedonic component varies according to the circumstances: What was unpleasant during the journey and disgusting on the atmosphere of a house becomes highly valuable when linked to health curative properties. This has been scientific proven by investigators, who reported that subjects rated a test odor more unpleasant when labeled as "body odor" than when labeled "cheddar cheese" (De Araujo et al., 2005).

There are many stinky cheeses but what is not common is to throw them away because of their smell. The smell of a camembert is not a fragrance we would like to spread throughout the entire house, but it does not repel its many lovers. In fact, a consumer would become suspicious in the presence of an odorless specimen.

What influences cheese odor? Basically, to prepare a cheese, milk is left to become sour, casein aggregates, a gel is formed, and whey is separated. So, at first, there is milk from cow, ewe, goat, buffalo, camel, or mare. Besides the animal, its feed influences the milk's flavor. Milk for cheese making can be raw or pasteurized. Renneting of the milk means the addition of enzymes, from animal (eg, extract of calf stomach) or from vegetal sources (eg, extract of *Cynara cardunculus* L.), acid, or both. Microorganism cultures, mainly starters of lactic acid bacteria, can be added to milk, which becomes especially important if milk has been pasteurized. The composition of starter and other microorganisms added depend on the type of cheese. Whey is separated (syneresis) and removed. With the exception of some fresh cheeses, NaCl is added. Ripening or maturation during storage is responsible to large biochemical, microbial, chemical, and physical changes, during which a great number of flavor compounds are formed. In some types of cheese, there are a flora of yeasts, bacteria, and molds developing on the rind (Walstra et al., 1999). Temperature and time on each stage of the process alter the final product and flavor. The rinds of some cheeses are sometimes washed often during ripening with brandy, port, beer, or salt water. The particularly pungent smell of the Vieux-Boulogne is attributed to its washing with beer.

## 3.3 ODOR AND CHEMICALS

Analytical instruments can be used to identify volatiles. However, not all volatiles are aroma active, or important to the human perception of odor. On the other side of the scale, we can find mercaptans, from which as few as 40 molecules can be enough to produce a strong emotional response.

It is difficult to classify odors based on chemical properties of molecules. Types of molecules within each odor quality can vary much in structure, and identical molecules

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can display significant odor differences. Enantiomers may or may not show differences in odor quality (Schiffman and Pearce, 2003).

Odor sensations are often produced not by a single compound but by mixtures where the number of compounds easily reach several hundreds. Human capacity to distinguish individual compounds from mixtures is however limited (Schiffman and Pearce, 2003).

Therefore, most odor sensations are produced by mixtures of compounds and cannot be attributed to a single one (Schiffman and Pearce, 2003). An extensive vocabulary exists of adjective descriptors, general, as well as specific, to a particular field of application.

## 3.4 ODOR EVALUATION

Consumers have their own odor evaluation system, and their own relation between their sensations and product quality. Their perception is a combination of physiological and memory responses (Croissant et al., 2011) but can by no means be ignored. Companies also have their sensory analysis, often mainly oriented to defect detection, but also with a component of satisfaction, and where the simple statement that the product smells to what it is, is by no means irrelevant. An inodorous product is regarded as junk food. Human judging, even when based on rigid protocols, is subjective. Attempts to turn sensory evaluation objective included the adoption of a sensory language, protocols for recruiting the panelists, and their training. Even so, people get tired and are influenced by their state of mind.

Scaling in sensory analysis is possible but it is generally product specific. The use of a universal intensity scale requires longer training (Croissant et al., 2011). The combination of human nose with gas chromatography (GC-O) allowed adding compound identity information in an attempt to understand their significance to the aroma. The human sniffer evaluates the aroma as it is eluted from the chromatographic column. The use of humans as sniffers leads to inconsistencies in smell perceptions, but there are other difficulties as detection limits are different for instrument and humans, possible coelution of poor resolved compounds, hot and dry gases at the exit of the GC detector dry the nasal mucosa, and the odorants from plastic components interfere (Schiffman and Pearce, 2003). Some corrections to these problems have been attempted by humidifying the gases and eliminating background odors.

## 3.5 ELECTRONIC NOSE

The electronic nose represents the ultimate attempt to have a human-independent evaluation of odor. It is intended to identify a mixture of compounds as a whole. It is not intended to identify individual chemicals. It is constituted by an array of sensors sensitive to the chemicals of interest, but that do not need to be selective. Mainly sensors based on metal oxide semiconductors (MOS), metal oxide semiconductor field effect transistors (MOSFET), conductive polymers (CP), but also acoustic sensors, bulk acoustic wave (BAW), usually also named quartz crystal microbalances, and surface acoustic wave (SAW), have been used for dairy product evaluation. Arrays including different transducers (hybrids) have also been assembled. The instrument is also composed of a sampler, or a sampling introduction system, and a data processing unit. Pattern recognition techniques have been employed to analyze electronic nose data. Statistical packages can be helpful for some common conventional statistical methods, such as principal component analysis (PCA), partial least square (PLS), discriminant function analysis (DFA), including linear discriminant analysis (LDA), cluster analysis, or artificial neural networks (ANN).

Most of the electronic noses that can be found in the literature do not fit into the previous description, and are composed of mass spectrometers hyphenated with gas chromatographs. The philosophy behind such instruments is much different, as they discriminate chemical compounds according to their elution time and the mass-to-charge ratio of the molecular ions and their fragments. It is unquestionable that after treating the results, differences in odors can be found, although many of the detected compounds can be irrelevant in terms of smell. Although more than 600 volatile compounds have been identified in cheese, the most abundant have little or no odor significance (Curioni and Bosset, 2002). Authors speak about the “hijacking” of the electronic nose designation from the mass spectrometers based systems (Mielle, 1996). Gardner and Bartlett (1994) defined an electronic nose as “an instrument, which comprises an array of electronic chemical sensors with partial specificity and an appropriated pattern recognition system, capable of recognising simple or complex odours.” In order to avoid misinterpretation, there are manufacturers of electronic noses, devices within the classical Gardner definition, who are changing the name of their instruments to “sensors array technology” (Mielle, 1996).

The ultimate performance of the device depends on all its components, and not only on sensors. They are, however, the heart of the device and must be carefully chosen. The previous knowledge on aroma chemical constitution and on their role in aroma perception is by no means irrelevant and should be considered on its development. Sometimes a compound is known to be linked to a specific process and for this particular application the electronic nose can be replaced by a single selective sensor for that target compound. There are also complex processes, as for instance rancidity development in butter, where, despite the many compounds evolved during the process and present in different ratios along the process, a single sensor sensitive to many of them was able to follow rancidity development and to detect distinct phases, perceived by sensory panelists (Gaspar and Gomes, 2012). However, it is not uncommon that an electronic nose is used when a marker compound has been identified, as a selective sensor is hard to develop and often not available (Bargon et al., 2003).

The choice of the sensors is critical as they must respond to those variables responsible for the differences between the specified classes, while for instance their sensitivity to humidity should be minimized, as well as drift along time. Too many sensors to classify a small number of samples is a dangerous situation to which Goodner et al. (2001) alerts, as overfitting can create artificial differentiation due to noise.

There are many different prototypes of artificial noses. However, consumers need a fully specified instrument, with formal specifications concerning analytical figures of merit as sensitivity, selectivity, repeatability, and methodologies (Mielle, 1996), available for most classical analytical instruments. Besides, consumers and inspectors want a “black box” able to automatically acquire and interpret data, in order to obtain a rapid classification of the product (Mielle et al., 2000). This means a tailored instrument that responds to selected questions such as: “Is this cheese made of ewe milk?” “Is this a S. Jorge cheese with a cure of 7 months?” “How bitter is this cheese?” “Is this cheese produced from raw or pasteurized milk?” “Is the rennet vegetal?” “Does this odor pattern conform to the specified denomination of origin, or is this a fraud?” while industry may want to test for differences in smell pattern induced by process changes, or look for defects.

### 3.5.1 Sample Introduction and Preconcentration

Handling and delivery systems can be static, or based on a controlled carrier gas which assures a constant flow through the entire instrument. Static systems are based on sensor readings after a steady-state value has been attained, while flow injection systems can use shorter cycles because equilibrium does not need to be reached, and most important, it needs not to be assured. Flow systems are most convenient for sensors, due to their simplicity and short measuring cycles.

Dairy samples have complex matrices, and, for electronic noses, solvent extraction has been replaced by headspace analysis. Headspace sampling can be performed by admitting a carrier gas to the sample container, which drives the vapor to the sensors. With short vapor pulses, it is possible to ignore changes in the concentration profile (Nakamoto, 2003). Static headspace involves the sample equilibration in a sealed container at controlled temperature and syringe withdraws through a septum. Dynamic headspace, also termed purge and trap (Sides et al., 2000), allows the continuously stripping of volatiles by an inert gas, and its enrichment in an inert trap. Porous traps such as Tenax can be used. Most convenient is the use of a solid-phase microextraction (SPME) fiber in the headspace of a vial containing a known amount of sample, at controlled conditions (temperature and stirring rate), for a defined period of time. Later compounds are thermal desorbed from the stationary phase coating the fused-silica fiber, and carried by a neutral flowing gas to the sensors. Although SPME could also be used in the direct extraction mode, their use in the headspace mode is generally necessary with high complex matrices that could damage the fiber. Fiber of appropriate polarity and film thickness should be selected, and mixed phase coatings are most appropriate. Pérès et al. (2001) compared four fibers of different composition used in the analysis of volatiles from Camembert, and concluded that Carboxen (CAR)/polydimethylsiloxane (PDMS) fiber showed particular affinity for the extraction of sulfur compounds and short fatty acids, while PDMS/divinylbenzene (DVD) fiber was more efficient than CAR/PDMS fiber to extract hexanoic and octanoic acids. DVD/CAR/PDMS fibers are difficult to produce and, according to Pillonel et al. (2002), they are sometimes delivered with visible fissures in the coating. Sample headspace should be kept as small as possible (Sides et al., 2000; Pillonel et al., 2002) in order to favor adsorption on the fiber and extraction yield. Temperature should be carefully controlled and chosen, in order to avoid artifacts.

### 3.5.2 Sensors and Sensors Layout

MOS, MOSFET, CP, BAW, or SAW sensors all have their advantages and drawbacks. Briefly, MOS and MOSFET sensors operate at high temperatures. MOS sensors can show a very good ratio of drift and lifetime in respect to sensitivity, but they lack selectivity, and above all, selectivities are not very different between different MOS sensors. Besides, they are subject to poisoning (Schaller et al., 1998), and precision is poor (Wilson and Baietto, 2009). Sensitivity of CP sensors is generally one order of magnitude lower than the sensitivity of MOS sensors (Wilson and Baietto, 2009), but their major drawback is their instability (Schaller et al., 2000). BAW or SAW sensors can be coated with a huge variety of materials, which makes them the best choice in terms of selectivity. Precision is good. However, acoustic sensors usually require higher volatile concentrations than the other sensor types (Schaller et al., 1998). Integration of different types of sensors can be appealing, but it is not much used.

Temperature or humidity changes may lead to drift. Causes for drift depend on the type of sensor. It may be wise not to include sensors with large variability on the array. A sensor with sensitivity to the compounds of interest close to zero but with significant noise should be discarded because it will degrade the array performance. On the other hand, the array should include sufficient sensor diversity in order to achieve the desired discrimination.

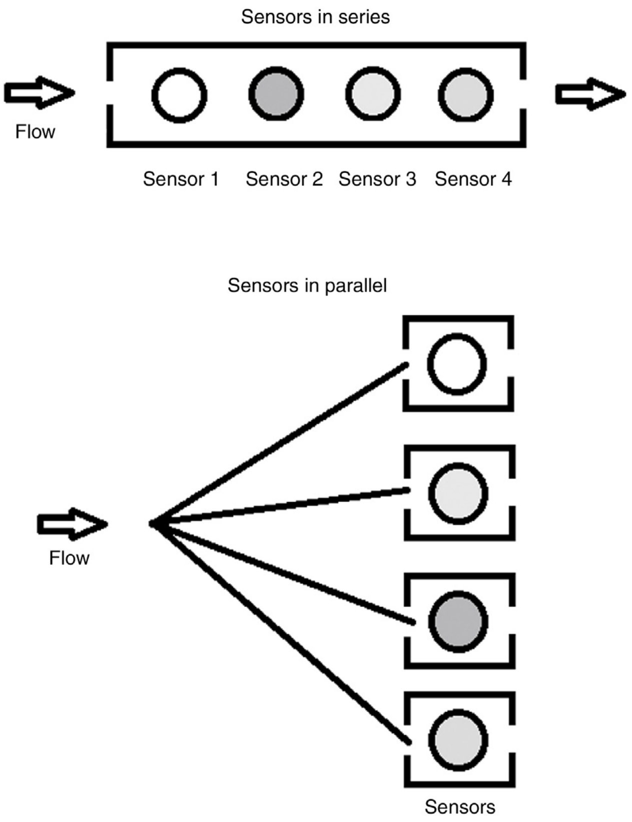

**FIGURE 3.1** **Sensors arrangement in series and parallel.**

Often all sensors are within the same cell and the flow carrying the sample will reach each sensor in sequence. Fig. 3.1 shows schematically such an arrangement and an alternative one, where each sensor is independently housed and the flow is divided and directed to each sensor. This parallel arrangement allows decreasing cell dimensions and optimizing its layout in order to increase sensitivity (Fernandes and Gomes, 2008). Sensitivity is generally regarded as independent of position (Nakamoto, 2003), but sensitivity of the sensors do depend on their position because flow division is often uneven as there are preferred positions dictated by flow constraints. The layout where sensors are located in a series is more common than the parallel arrangement, and probably is the layout found in most devices whenever nothing is said about layout.

## 3.6 ELECTRONIC NOSES FOR DAIRY PRODUCTS: AN OVERVIEW

Table 3.1 lists a wide selection of the most representative electronic noses applied to dairy products and published in the literature.

Quality control must start with milk analysis, or even before, by inspecting animal health. A sampling method to collect the breath of cows has been reported by Elliott-Martin et al. (1997). Those samples were analyzed by a commercial electronic nose, with six MOS sensors, and classification of cows as healthy or ketotic was correctly predicted in 34 out of 38 samples. Electronic noses have also been developed to detect bacterial contamination in milk (Magan et al., 2001; Sivalingam and Rayappan, 2012; Ali et al., 2003; Korel and Balaban, 2002), milk aging, and other problems responsible for off-flavor (Sivalingam and Rayappan, 2012), not always successfully (Ali et al., 2003). An electronic nose was proposed to determine milk shelf life, based on bacteria growth (Labreche et al., 2005). Mastitis is common in dairy cows and Eriksson et al. (2005) used a hybrid commercial e-nose based on MOSFET, and MOS sensors, and one IR $CO_2$ sensor, to discriminate between milk from healthy and mastitic cows. Rancid milk (Capone et al., 2000, 2001) and also the separation in UHT and pasteurized milk (Capone et al., 2001) have been achieved with four or five $SnO_2$ sensors, although eight BAW coated with porphyrins (Di Natale et al., 2000) were also able to detect spoiled milk but not to distinguish between UHT and pasteurized milk samples. Separation of UHT commercial milk samples in normal and anomalous odor was achieved by 10 MOS sensors and linear discriminant analysis (Brambilla and Navarotto, 2010). Seasonal variation of milk has also been discriminated (Biolatto et al., 2007), as well as synthetic milk flavorings from natural milk flavorings and enzyme-induced milk flavoring (Wang et al., 2010). An electronic nose was able to recognize milk products from a particular dairy and products with different fat content (Brudzewski et al., 2004). Electronic noses have also been used in quality control of specific products, for instance, to assure that the aroma from formula milk is not significantly different from the aroma of breast milk (Li et al., 2009). A commercial electronic nose was used not only to discriminate milks but also culture dairy products (Collier et al., 2003). Block milk is obtained by heating and drying mixtures of milk and sugar and it is used in the production of chocolate. Flavor components are formed via Maillard reactions and flavor depends on the processing. A commercial electronic nose composed of 12 conductive polymer-coated sensors was used to distinguish between different intermediate products obtained during the block milk processing (Zondervan et al., 1999).

Cultured cream butter, sweet cream butter, and cultured butter from sweet cream were differentiated by an electronic nose composed of 10 MOS sensors (Lorenzen et al., 2013). A different approach was used to follow butter rancidity because a single BAW-coated sensor, sensitive to all the volatile compounds identified by GC/MS (Gaspar and Gomes, 2012), was much more effective on following the different stages of butter deterioration. The success of the sensor, apart from its sensitivity to all the compounds of interest, is mostly due to its relative sensitivities to the evolved compounds, since it is more sensitive to minor

**TABLE 3.1** Overview of Electronic Noses Collected from the Literature

| Sensor Type | No. of Sensors | Sample Introduction | Layout | Commercial | Data Processing | Purpose | References |
|---|---|---|---|---|---|---|---|
| MOS | 6 | Samples collected by an especially designed breath sampler | Series | Yes | PCA, CA | Discriminate cows with ketosis | Elliott-Martin et al. (1997) |
| CP | 14 | Headspace flushed | — | Yes | DFA, CA, PCA, ANN | Separate unspoiled from microbial spoiled milk | Magan et al. (2001) |
| MOS | 4 | Sensors 3 cm above milk surface | | No, Taguchi sensors and a ZnO home-made | PCA | Milk quality control by detecting off-flavor | Sivalingam and Rayappan (2012) |
| BAW | 6 | Headspace sampling by plunging a syringe | Series | No | PCA | Discriminate contaminated milk | Ali et al. (2003) |
| CP | 12 | Headspace | — | Yes | DFA | Classification of milk according to microbial counts | Korel and Balaban (2002) |
| MOS | 18 | Headspace injection | — | Yes | Norms of sensor responses, PCA | Milk shelf life | Labreche et al. (2005) |
| CP | 28 | Headspace and stopped flow | — | Yes | LDA | Grouping milks by the season | Biolatto et al. (2007) |
| MOSFET, MOS, IR $CO_2$ | 10 MOS-FET, 12 MOS, 1 IR | Headspace after incubation at 60°C | — | Yes | DPLSR, ANN | Separation of healthy milk from mastitic cow milk | Eriksson et al. (2005) |
| MOS | 4 or 5 | Headspace stripping | Series | No | PCA | Separation of UHT and pasteurized milk; separation by rancidity levels | Capone et al. (2000, 2001) |
| BAW | 8 | Headspace injection | Series | No | PCA | Separation of spoiled milk samples; not able to separate UHT and pasteurized milk | Di Natale et al. (2000) |
| MOS | 10 | Headspace flushed | — | Yes | PCA, LDA | Separate UHT milk in normal and anomalous odor | Brambilla and Navarotto (2010) |
| MOS | 7 | Headspace flushed | Series | No, Taguchi sensors | PCA, SVM neural network | Separate milk from different dairies, and by fat content | Brudzewski et al. (2004) |
| MOS, CP | 16 (11 selected) | Headspace injection | — | Yes | PCA | Evaluate difference in aroma between formula and breast milk | Magan et al. (2001) |
| MOS | — | Headspace injection | — | Yes | CA | Discriminate among milks and among yogurts | Collier et al. (2003) |

*(Continued)*

**TABLE 3.1** Overview of Electronic Noses Collected from the Literature (*cont.*)

| Sensor Type | No. of Sensors | Sample Introduction | Layout | Commercial | Data Processing | Purpose | References |
|---|---|---|---|---|---|---|---|
| MOS | 18 | Headspace injection | Series in three chambers | Yes | PCA | Differentiate between synthetic, natural, and enzyme milk flavoring | Wang et al. (2010) |
| CP | 12 | Dynamic headspace | — | Yes | ANN, PCA | Distinguish different intermediate products during block milk processing | Zondervan et al. (1999) |
| MOS | 10 | Headspace flushed | — | Yes | PLS-LDA | Differentiate butter type | Lorenzen et al. (2013) |
| BAW | 1 | SPME | Parallel | No | Response plot | Rancidity of butter | Gaspar and Gomes (2012) |
| BAW | 2 | SPME | Parallel | No | Response plot | Discriminate milk origin and animal renneting | Pais et al. (2015) |
| MOS | 6 | Headspace flushed | Sensors in one cell | No, Taguchi sensors | DFA | Discriminate milk origin | Haddi et al. (2010) |
| BAW | 4 | SPME | Parallel | No | Dendrogram/ response plot | Ewe milk cheese, mozzarella, Flamengo and Brie, Gruyère | Pais et al. (2012) |
| MOS | 12 | FIA | — | Yes | PCA | Modeling ripening | Trihaas et al. (2005) |
| CP | 14 | FIA | — | Yes | PCA | Classification in ripening stages | Trihaas and Nielsen (2005a,b) |
| MOS | 8 (3 optimum number) | Headspace injection | — | Yes | PCA | Classification in commercial cheeses—grader classes | O'Riordan and Delahunty (2003a,b) |
| BAW | 12 (1 uncoated), 6 are enough | Volatiles were pumped | Series | No | — | Ripening stage | Bargon et al. (2003) |
| MOSFET and MOS | 10 MOSFET, 12 Taguchi | Headspace injection | Series | Yes | PCA, LDA | Separation into fresh, aged, and very aged classes | Benedetti et al. (2005) |
| BAW, MOSFET, CP, MOS | 12 CP + 8 MOS, 6 BAW, 10 MOSFET + 5 MOS | — | — | Yes, MOS sensors were the most sensitive, but suffer from poisoning. | — | Ripening stage | Schaller et al. (1999) |
| MOS | 6 | Headspace injection | — | Yes | ANN | Classification of Pecorino cheeses according to ripening and manufacture technique | Cevoli et al. (2011) |
| MOS | 6 | Headspace flushed | Series | Yes | CA | Test for differences in cheeses when ewes were fed with linseed enriched diets | Branciari et al. (2012) |

volatiles that become important at a later stage of rancidity development, than to the most abundant compounds that increase significantly their concentration at the right beginning of the process. Being composed of a single sensor, it cannot be called an electronic nose.

Cheeses are produced from milk from different animals, and even from mixed milks from two animal species. Ewe milk cheeses tend to be more expensive than cow milk cheeses and there is a need to detect frauds. An electronic nose based on two piezoelectric quartz crystals coated with Carbowax 20M and copper phthalocyanine, respectively, was able to distinguish cheeses produced from ewe, goat, or cow milk (Pais et al., 2015). This discrimination is based on the different composition of volatiles that were adsorbed on an SPME fiber and afterward desorbed in an oven and carried by a nitrogen flow to a valve which split and distributed the gaseous stream to the sensors. A conventional gas chromatogram of the volatiles desorbed from the SPME fiber could be used to make the same discrimination after plotting the first and third principal components obtained from the areas of the peaks corresponding to 50 detected compounds (Gomes et al., 2014). An array of six Taguchi MOS sensors has already been used with success to discriminate cheeses made from goat and cow milk and from mixtures (Haddi et al., 2010). In order to evaluate the shelf life of Crescenza cheese, an Italian soft cheese, an electronic nose with 22 sensors, 10 MOSFETs, and 12 Taguchi MOS sensors was used with linear discriminant analysis (LDA) to classify cheeses into fresh, aged, and very aged (Benedetti et al. 2005).

Cheese can be produced from raw milk, or from pasteurized milk, which offers extra protection to the consumer. Serra da Estrela cheese, a renowned Portuguese cheese bearing the status of "protected designation of origin (PDO)," is produced from raw milk without the addition of any starter and it is sold after a typical ripening period of 45 days at temperatures of 10°C, conditions at which most pathogen microorganisms cannot survive. Although no health problems have been registered due to this cheese consumption, the theme is controversial and the cheese faces some constraints to be consumed in Brazil, where cheeses from raw milk cannot be produced. Attempts to produce this cheese from pasteurized milk results in a product organoleptically less appealing (Macedo et al., 1993), which does not hold the PDO label. The content of several ketones, and among them 2-heptanone, aldehydes, and sulfur compounds has been shown to increase with heat treatment of cow's milk (Hougaard et al., 2011). However, the volatile profile of a farmhouse Halloumi-type cheese produced with raw milk was enriched in relation to the cheese made from pasteurized milk and the majority of volatiles, except for the previously mentioned compounds, namely aldehydes and sulfur compounds, were more abundant. The enhanced volatile compounds were mainly acids, alcohols, and esters (Hayaloglu and Brechany, 2007).

Important changes in flavor occur during cheese maturation (McSweeney and Sousa, 2000; McSweeney, 2004). Flavor and texture depends on the breaking down of fat and protein by complex biochemical reactions. The extent of changes depends on pH, moisture, salt, temperature, and starters present. Cheeses from raw milk ripen more quickly than cheeses from pasteurized milk (Cabezas et al., 2007). Besides, there is a geographical variability in microbial composition of cheeses produced from raw milk (Cabezas et al., 2007). Trihaas et al. (2005) and Trihaas and Nielsen (2005a,b) followed the volatile composition of Danish blue cheese during ripening and tried to classify the cheeses by ripening stage, both by GC/MS and with an electronic nose. The authors used commercial e-noses, either based on 12 MOS sensors (aFOX-300) (Trihaas et al., 2005), or 14 conducting polymers (BH-114: Bloodhound Sensors Lda) (Trihaas and Nielsen, 2005a,b). Analyses on the e-nose were conducted by flow injection. The classification is complicated due to the fact that volatiles are both being produced and decomposed during ripening, but classification was as successful with the e-nose based on the conducting polymers as with the GC/MS data. Bargon et al. (2003) followed the ripening stage of Emmental cheese, which they associate to the evolution of 2-heptanone, with an electronic nose composed of 12 BAW sensors, one of them uncoated and used as a reference. One of the sensors is particularly sensitive to the target compound, and six sensors are enough to discriminate between Emmental cheese ripened at 3, 8, and 12 months. Previously, Schaller et al. (1999) have tested some commercial electronic noses to discriminate Emmental cheeses with different ripening time, and BAW sensors used were insensitive to the differences. The MS system showed a very low sensitivity. MOSFET or CP sensors did not give good discrimination. The most efficient sensors were the MOS sensors, in spite of suffering from poisoning. Schaller and coworkers complained about the many technical problems of the available instruments. Pecorino cheeses have been classified according to their ripening time and manufacturing techniques by a commercial array of six MOS sensors and an artificial neuron network (Cevoli et al., 2011). Cevoli and coworkers claimed to have obtained best results with the e-nose rather than with SPME-GC/MS. No differences in Peccorino cheese volatiles produced from milk of ewe fed with a normal diet and with a diet enriched with extruded linseed have been detected by an electronic nose composed of six MOS sensors (Branciari et al., 2012).

Milk coagulation can be made by adding an acid, or a rennet. Rennet can have an animal origin, like the one obtained from the calf stomach, or a vegetable origin, like the one obtained from the thistle flowers of *Cynara* L., or a microbial origin. Proteolytic activity of those rennets varies and even differs according to the animal species from which the milk comes (Macedo et al., 1993). Moreover, the enzymes vary in activity among the thistle species (Fernández-Salguero and Sanjuán, 1999). Pais et al. (2015) succeeded in separating cheeses made with animal rennet from cheeses made with other rennets, with the electronic nose used to separate cheeses according to the milk origin. Coagulation kinetics can be also followed with an uncoated piezoelectric quartz crystal (Pais et al., 2015).

GC-O analysis of several cheeses showed that only a small number of the many volatile compounds contributes significantly to their flavor (Curioni and Bosset, 2002). It has been pointed out that the main differences among cheese varieties is related to quantitative differences, but not related to the concentration of a particular compound, rather than on a balance or weighted ratio of the components (Curioni and Bosset, 2002). Pais et al. (2012) were able to distinguish ewe milk made cheeses from the others with two sensors, whereas two other sensors could separate three classes of cheeses: mozzarella, Flamengo and Brie, and Gruyère, among several other cheeses, irrespectively of the place where they have been produced. Ten BAW sensors were used in this work; however, at last, it was found that only four of them were useful. Shredded-type pizza cheese, Cheddar cheese, mozzarella block cheese, and white mold-ripened cheese were separated by applying a principal component analysis to the data obtained by mass spectrometry (Hong et al., 2012).

O'Riordan and Delahunty (2003a,b) used an electronic nose to classify Cheddar cheeses in classes consistent to grader commercial practices.

## 3.7 CONCLUSIONS

There is a great interest for handheld instruments that respond to simple questions posed by food inspectors, general consumers, and the dairy industry. Commercial electronic noses are designed for general-purpose use and besides selectivity and sensitivity of the sensors in the array do not match the needs for a particular application. They include redundant sensors, and systems are not fully automated, with data processing and statistical analysis to be performed by the users. This prevents their widespread use, due to lack of successful discrimination of samples and the need of skilled operators.

Although the ideal situation would be to have dedicated instruments to perform a particular task, a review of the scientific literature shows that, even among scientific investigators, most used instruments that are commercial, and there are not many scientists building tailor-made e-noses. Not surprisingly, Taguchi sensors dominate the applications. Some authors blame the lack of interest of the dairy industry to be only interested in mass production and not interested in the food industry (Mielle, 1996).

Chemical sensors' complexity is obvious when we compare with physical sensors and look for their widespread in industry and for personal use. Even the most successful sensor in the chemical history, the pH electrode, needs frequent calibration. There is a lack of a variety of long-term reliable sensors, with low power consumption and with sensitivity and detection limits at the level of the human nose. The obvious conclusion is that electronic noses did not reach their maturity in terms of technological development. Mielle's concerns, as expressed in 1996, that this maturity may not be reached in a short time, is still to come (Mielle et al., 2000). In spite of the increasing number of publications with electronic noses devoted to dairy product classification, there is still no cheap, reliable, foolproof instrument available.

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## Chapter 4

# Coffee and the Electronic Nose

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## 4.1 INTRODUCTION

Coffee is produced from ground-roasted beans with an aroma and taste that makes it one of the most popular beverages in the world (De Maria et al., 1999). In terms of financial value, coffee is the most important agricultural commodity after petroleum (Sunarharum et al., 2014). An interesting feature of coffee is the fact that the beverage does not have relevant nutritional value, being consumed basically for the stimulatory effect, related to the presence of caffeine, and for its pleasing aroma and taste (Grosch, 2001). Besides these two fundamental attributes, aroma remains the most important consumer parameter and warrants thorough investigation from a sensory and compositional perspective (Sunarharum et al., 2014).

## 4.2 COFFEE VOLATILE COMPOSITION

The aroma of coffee is formed by an extremely complex mixture of numerous volatile compounds that exhibit many qualities, different levels of intensity, and different concentrations. In this way, the contribution of each of these volatiles to the final aroma of coffee is quite varied and may also occur as antagonistic and synergistic interactions between these different compounds (Moreira et al., 2000). The chemical composition of the green coffee bean is quite complex and dependent on several factors, such as species, cultivars, provenance (climate, soil type, altitude, etc.), and agronomic practices (Farah, 2009; Link et al., 2014). Table 4.1 illustrates the difference between the chemical composition of the species *Coffea arabica* and *Coffea canephora*, which are predominantly used by industry. The compounds already present in the bean will be precursors for new compounds, which are extremely important for the typical aroma of the final beverage (De Maria et al., 1996).

The aroma of green coffee beans is very weak and even difficult to be detected by an electronic nose (Rodríguez et al., 2010). Therefore, roasting is fundamental to obtain the typical coffee aroma. Different time–temperature histories lead to distinct aroma compound profiles, thus a precise control of roasting parameters is required (Schenker et al., 2002). Roasting is induced into the green beans by heat energy, hot gases, or hot metallic surfaces from the roaster. The first roasting stage is a drying phase, evidently endothermic, during which moisture is eliminated. A majority of aroma compounds show the highest increase in concentration with bean water content from 2 to 7% (wb). The smell of the beans changes to bread-like and the color turns yellowish. In the second stage, the actual roasting phase, a number of complex pyrolytic reactions starts at 160°C and peaks at 210°C. These reactions are interrupted at the desired point based on the color of the bean or the programmed time. The chemical composition of the beans is drastically modified, with the release of large amounts of carbon dioxide and the formation of many hundreds of volatile substances. The ground roast coffee may be available on the market in different roasting degrees, varying in color from very light to very dark (Buffo and Cardelli-Freire, 2004; Eggers and Pietsch, 2001; Farah, 2009). Some of the major volatile groups that impact the aroma of roasted coffee and their respective precursors are presented in Table 4.2.

Studies on the role of volatiles are very complex because different concentrations of the same component could lead to diverse sensory characteristics. Therefore, the determination of the concentration and perception thresholds of volatile compounds in coffee has been shown to be an arduous task (Mello and Trugo, 2003). The main chemical reactions that occur during roasting include Maillard reactions (nonenzymatic browning); phenolic acid and carotenoid degradation; Strecker degradation; breakdown of sulfur amino acids, hydroxy-amino acids, proline, and hydroxyproline; degradation of trigonelline, chlorogenic acids, quinic acid, pigments, and lipids; as well as reactions between other intermediate products (Sunarharum et al., 2014). Roasting the beans from light to dark increases the sulfurous/roasty, earthy, and smoky notes in the aroma profile (Grosch, 2001). Due to variability and the amount of parameters that interfere with the aroma of coffee, it is not surprising that more than 800 different compounds have already

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**TABLE 4.1** Chemical Composition of the Nonvolatile Fraction of Green Coffee Beans (Farah, 2009)

| Component | Content[a] (g/100g Dry Basis) | |
|---|---|---|
| | *Coffea arabica* | *Coffea canephora* |
| **Carbohydrates and Fibers** | | |
| Sucrose | 6.0–8.0 | 4.0 |
| Reducing sugars | 0.1 | 0.4 |
| Polysaccharides (arabinogalactan, mannan, and glucan) | 34–44 | 48–55 |
| Lignin | 3.0 | 3.0 |
| Pectins | 2.0 | 2.0 |
| **Nitrogenous Compounds** | | |
| Protein | 10.0–11.0 | 11.0–15.0 |
| Free amino acids | 0.5 | 0.8 |
| Caffeine | 0.9–1.2 | 1.5–2.5 |
| Trigonelline | 0.8–2.0 | 0.6–0.7 |
| **Lipids** | | |
| Coffee oil (triglycerides with unsaponifiables) | 16.0 | 10.0 |
| Diterpene esters | 0.9 | 0.2 |
| Minerals (41% K and 4% P) | 3.0–4.2 | 4.4–4.5 |
| **Acids and Esters** | | |
| Total chlorogenic acids | 4.1–7.9 | 6.1–11.3 |
| Aliphatic acids | 1.0 | 1.0 |
| Quinic acid | 0.4 | 0.4 |

[a] *Content varies with cultivars, cultivation climate, soil, methods of analysis, etc.*

been identified (De Maria et al., 1999; Moreira et al., 2000). But it is noteworthy that none of these molecules can alone be identified as a marker (Pardo et al., 2000). Furthermore, the diverse methods used for measuring volatile composition may also result in differences between key volatiles of any particular coffee sample (Sarrazin et al., 2000; Sunarharum et al., 2014). Several tables can be found in literature with listing key odorants (Grosch, 2001). For example, Table 4.3 presents the important volatile compounds in medium-roasted arabica coffee blends from Colombia.

The production and sales of instant coffee have increased markedly in most countries. From the roasted coffee, extraction columns are used in the industrialization of soluble coffee to obtain the water-soluble coffee extract. The soluble solids' content of the extract of coffee is relatively low, and therefore, for economic reasons, it is preconcentrated in an evaporation process, and subsequently, the remaining water is removed by spray-drying or by freeze-drying. The research for the approximation of the soluble coffee aroma to the brewed coffee has been constant. In general, the goal is to increase the aroma intensity, and for this purpose, the feasibility of using electronic noses has already been studied (Clarke, 2001). Eighty-eight important aroma compounds were identified in the soluble coffee using gas chromatography and mass spectroscopy (GC–MS). Methional, furaneol (4-hydroxy-2,5-dimethyl-3-furanone), 2-furfurylthiol, and isovaleric acid (3-methylbutanoic acid) are among the recognized components (Viegas and Bassoli, 2007). There are also other highly volatile molecules whose descriptive sensory is evaluated as a sweetish/caramel group such as 2,3-butanedione and 2,3-pentanedione. Strecker degradation leads to the formation of several of the compounds reported as important in the formation of

**TABLE 4.2** Volatile Groups of Impact in the Aroma of Roasted Coffee (Mello and Trugo, 2003)

| Volatile Group | Precursors | Example |
|---|---|---|
| Pyrroles and alkyl pyrroles | • Amino acids + carbohydrates<br>• Hydroxy-amino acids | *N*-methylpyrrole |
| Furanones | • Sucrose | Dihydro-2-methyl-3(2H)-furanone |
| Furaldehydes | • Sucrose<br>• Arabinogalactans | 2-Furaldehyde |
| Alkyl furans | • Arabinogalactans<br>• Amino acids + carbohydrates | 5-Methyl-2-vinyl furan |
| Pyrazines and alkyl pyrazines | • Hydroxy-amino acids | Methylpyrazine |
| Acyl pyrroles | • Amino acids + carbohydrates | 2-Acetyl-1-methylpyrrole |
| Carbocyclic compounds | • Sucrose | 3-Methyl-1,2-cyclopentanedione (Cicloteno) |
| Pyridines | • Trigonelline<br>• Hydroxy-amino acids | Pyridine |
| Phenols | • Chlorogenic acid | Phenol |

the aroma of instant coffee, such as 2-methylbutanal and 3-methylbutanal, which are sensorially perceived even in high dilutions. Many pyrazines resulting from Maillard reactions were identified in this same work, and among them, one can cite 2,3-dimethyl-pyrazine and 2,6-dimethylpyrazine.

The GC-MS is by far the most popular technique for the identification of volatile compounds in coffee, but this methodology is very expensive and laborious (Rodríguez et al., 2010). Furthermore, compositional data alone is not enough to explain the importance of key compounds and the nature of their contribution. Similarly, sensory information of coffee aroma properties, in the absence of good quality chemical data, cannot be used to explain specific sensory attributes. Good quality and comprehensive research that matches these properties in coffee to explain the compositional basis of coffee aroma is still limited. To fully understand the correlation between sensory data (panel of experts) and analytical measurements, researchers may use multivariate data analysis tools. Despite the wide application of multivariate techniques, to correlate compositional data with sensory attributes is an arduous task. This problem becomes more difficult if the methodology used to collect the information is not sufficiently comprehensive and without a high degree of accuracy and precision. Consequently, there are few studies to date that correlate physicochemical and sensory attributes of coffee aroma by means of multivariate tools (Farah et al., 2006; Sunarharum et al., 2014). Therefore, a quantitative evaluation and rational design method has been needed for the consumer-oriented development of coffee drink products (Michishita et al., 2010).

**TABLE 4.3** Groups of Volatile Compounds with Similar Odor Qualities: Concentrations in Medium-Roasted Arabica Coffee Blends from Colombia (Grosch, 2001)

| Group/Odorant | Concentration (mg/kg) | |
|---|---|---|
| | *Mean* | *Variation*[a] |
| **Sweetish/Caramel Group** | | |
| Methylpropanal | 28.2 | 24.0–32.3 |
| 2-Methylbutanal | 23.4 | 20.7–26.0 |
| 3-Methylbutanal | 17.8 | 17.0–18.6 |
| 2,3-Butanedione | 49.4 | 48.4–50.8 |
| 2,3-Pentanedione | 36.2 | 34.0–39.6 |
| 4-Hydroxy-2,5-dimethyl-3(2H)-furanone | 120 | 112–140 |
| 5-Ethyl-4-hydroxy-2-methyl-3(2H)-furanone | 16.7 | 16.0–17.3 |
| Vanillin | 4.1 | 24.0–32.3 |
| **Earthy Group** | | |
| 2-Ethyl-3,5-dimethylpyrazine | 0.326 | 0.249–0.400 |
| 2-Ethenyl-3,5-dimethylpyrazine | 0.053 | 0.052–0.053 |
| 2,3-Diethyl-5-methylpyrazine | 0.090 | 0.073–0.100 |
| 2-Ethenyl-3-ethyl-5-methylpyrazine | 0.017 | 0.015–0.018 |
| 3-Isobutyl-2-methoxypyrazine | 0.087 | 0.059–0.120 |
| **Sulfurous/Roasty Group** | | |
| 2-Furfurylthiol | 1.70 | 1.6–1.70 |
| 2-Methyl-3-furanthiol | 0.064 | 0.060–0.068 |
| Methional | 0.239 | 0.228–0.250 |
| 3-Mercapto-3-methylbutyl formate | 0.112 | 0.077–0.130 |
| 3-Methyl-2-buten-1-thiol | 0.0099 | 0.0082–0.013 |
| Methanethiol | 4.55 | 4.4–4.7 |
| Dimethy trisulfide | 0.028[b] | |
| **Smoky/Phenolic Group** | | |
| Guaiacol | 3.2 | 2.4–4.2 |
| 4-Ethylguaiacol | 1.6 | 1.42–1.8 |
| 4-Vinylguaiacol | 55 | 45–65 |
| **Fruity Group** | | |
| Acetaldehyde | 130 | 120–139 |
| Propanal | 17.4[b] | |
| (E)-β-damascenone | 0.226 | 0.195–0.260 |
| **Spicy Group** | | |
| 3-Hydroxy-4,5-dimethyl-2(5H)-furanone | 1.58 | 1.36–1.90 |
| 4-Ethyl-3-hydroxy-5-methyl-2(5H)-furanone | 0.132 | 0.104–0.160 |

[a] *Lowest and highest values of the samples.*
[b] *Only one sample was analyzed.*

## 4.3 COFFEE DATA ANALYSIS FOR ELECTRONIC NOSE

The electronic nose classifies and discriminates the aroma by statistical analyses of sensor resistances (Michishita et al., 2010). Therefore, once the data from the individual sensors from the array is collected, the electronic nose systems require a suitable pre- and postprocessing procedure (Pardo and Sberveglieri, 2005). Apart from outlier detection, data preprocessing consists of normalization, possibly some ad hoc data processing (eg, drift compensation), and feature/parameters extraction or selection (Pardo et al., 2000). Diverse features have been extracted from the sensor array signals, including steady-state (fractional change, relative, difference, and log) and transient (Fourier and wavelet descriptors, integral and derivatives) parameters (Distante et al., 2002) and even parameters extracted from the phase space (ie, the space formed by the time response and its first derivative; Falasconi et al., 2005). The last feature could be interesting because it takes into account both static and dynamic information at the same time (Martinelli et al., 2003). In addition, feature extraction can produce consistent data for the pattern recognition process (Wang et al., 2009). In postprocessing, techniques of pattern recognition and classification include principal component analysis (PCA), linear discriminant analysis (LDA), *k*-nearest-neighbor (k-NN), partial least squares (PLS), discriminant function analysis (DFA), cluster analysis (CA), fuzzy logic (FL), artificial neural network (ANN), support vector machines (SVM), and relevance vector machines (RVM). Among these techniques, PCA, PLS, LDA, DFA, and CA are based on a linear approach; whereas FL, ANN, SVM, and RVM are regarded as nonlinear methods (Loutfi et al., 2015).

In pattern recognition, the k-NN is a nonparametric method used for density estimation and can be extended to the problem of classification. The input consists of the *k* closest training examples in the feature space and the output is a class membership. A sample is classified by a majority vote of its *k* nearest neighbors (Bishop, 2006). The k-NN was employed as a feature selection to analyze roasted coffee ripening (Falasconi et al., 2005).

PCA is a nonsupervised method capable of reducing data dimensionality by grouping highly correlated information into a new axis system, then finding groups of samples. This analysis mathematically transforms the sensor array data into orthogonal components, known as principal components (PC), which are formed by two matrices known as scores and loadings. Scores are projections of samples on the new axis, while loadings have the weight information to transform the original variable in scores (Wold et al., 1987). To the electronic nose, dimensionality reduction is important since working with a smaller dimension means a drastic reduction in the number of operations during postprocessing. For this purpose, PCA was applied in coffee analysis (Pardo and Sberveglieri, 2002, 2005; Romani et al., 2012; Wang et al., 2009) and for clustering evaluation (Benedetti et al., 2004; Bona et al., 2012; Brudzewski et al., 2012; Pardo and Sberveglieri, 2002; Rodríguez et al., 2010; Ulmer et al., 1997); to select suitable sensors (Kermani et al., 2005; Michishita et al., 2010); to feature selection (Pardo et al., 2000); and to optimize the sampling conditions (ie, amount of sample, vial volume, and equilibration time for headspace generation; Falasconi et al., 2005).

LDA searches for those vectors in the underlying space that best discriminate among classes (rather than those that best describe the data). More formally, given a number of independent features relative to which the data is described, LDA creates a linear combination of these, which yields the largest mean differences between the desired classes (Martínez and Kak, 2001). In coffee analysis, LDA was applied to classify beans from different countries (Michishita et al., 2010).

Machine learning (ML) techniques are a set of techniques based on several statistical principles to perform tasks of regression and pattern recognition (Bishop, 2006). Among them, the artificial neural networks (ANNs) approach can represent complex and nonlinear input–output relationships. ANNs have been the target of recent research in several areas; Haykin (2008) presents a comprehensive text on the subject, covering both its implementation and its application. Several types of ML techniques were applied, mainly in classification tasks, for coffee analysis including multilayer perceptron (MLP; Bona et al., 2011; Kermani et al., 2005; Pardo and Sberveglieri, 2002; Pardo et al., 2000; Rodríguez et al., 2010; Romani et al., 2012; Ulmer et al., 1997); general regression neural network (GRNN; Romani et al., 2012); support vector machines (SVM; Brudzewski et al., 2012; Pardo and Sberveglieri, 2005); relevance vector machines (RVM; Wang et al., 2009); and fuzzy neural networks (FNN; Singh et al., 1996). There are also applications of ANNs for cluster analysis using self-organizing maps (SOM; Bona et al., 2012) and to correlate sensory evaluation with an electronic nose using MLP (Michishita et al., 2010; Pardo and Sberveglieri, 2002).

## 4.4 ELECTRONIC NOSE APPLICATIONS IN COFFEE ANALYSIS

The electronic nose has been frequently used for volatiles analysis in foods (Deisingh et al., 2004; Ghasemi-Varnamkhasti et al., 2010; Loutfi et al., 2015). Due to the complexity of the coffee aroma, already described in the previous section, a variety of applications of the electronic nose have been carried out in past years.

Actually the evaluation of the degree of coffee roasting is mainly based on the empirical final color observation, thus it requires well-trained operators with a high degree of skill. A portable e-nose (PEN2; Airsense Analytics, Germany) composed of an array of 10 temperature-moderated metal oxide sensors (MOS) was tested as a possibility to the roasting process automation and to set up a more reproducible procedure for final coffee bean quality characterization (Romani et al., 2012). The PEN2 was combined with an ANN to evaluate and predict different roasting degrees on the basis of their flavor release. In addition, e-nose data computed with ANN were used to build up provisional models for some coffee characteristics (ie, weight loss, moisture, density, and color) that are traditionally used to evaluate the roasting degree. Different roasting degrees were predicted with good accuracy showing high prediction capability for both roasting time and coffee quality parameters. In a previously work, an array of 12 different commercial tin oxide gas sensors with partially overlapping sensitivities was used to evaluate the effect of different roasting times (Gardner et al., 1992). Using a DFA method, the samples were relatively well segmented by degree of roasting. Moreover, the authors used the same e-nose to classify three commercial types of coffee applying DFA for pattern recognition with up to 90% of correct classification. In a subsequent work, the same data set was reevaluated and the classification accuracy was improved using an FNN (Singh et al., 1996).

After the roasting and before the packaging step, the coffee grains could be stored in batches within suitable silos to undergo a process named blend ripening or seasoning, which modifies the blend aroma. In order to monitor the coffee quality, an expert coffee taster evaluates the beans each day by smelling and drinking a cup of coffee. The Electronic Olfactory System EOS$^{835}$ (SCAMI Imola, Italy), equipped with six MOS sensors, was used to evaluate blends made by 12 different types of monocultivar arabica coffee during seasoning (Falasconi et al., 2005). The results showed that sampling conditions like vial preparation (ie, headspace volume and coffee quantity), headspace generation time, and even the variation of environmental conditions (ie, samples drawn out in the afternoon or in the morning) strongly influenced the correct segmentation of the samples according to the ripening period. It also showed that feature selection is very important because it improves the classification. The results obtained bring stronger evidence that the feature extracted in phase space leads to the best performance. Besides, the electronic nose, after optimization of the sampling parameters and suitable data processing, can be used to monitor the coffee blend during the seasoning process for evaluating the optimal ripening time.

The pattern recognition of coffee aroma is another important application of the electronic nose, and several works have been published since 1991 (Aishima, 1991). An electronic nose with 32 MOS sensors (AromaScan plc., United Kingdom) was applied to 6 sets of popular coffee varieties in the USA (Colombian, Turkish, Arabian with southern pecan flavor, Arabian with caramel flavor, Arabian with apricot flavor, and Arabian with orange flavor; Kermani et al., 2005). In fact, not all of the sensors respond exclusively to the odorants of interest; therefore, some of the sensors can be eliminated from the computations without a major loss of information. For these reasons, the authors employed a feature extraction technique by PCA. Afterwards, an MLP trained with the Levenberg–Marquardt method and parameters optimized using a genetic algorithm was employed to classify the coffees using the electronic nose data. For the test set, 98% of the samples were correctly classified showing that the electronic nose is a reliable and fast tool to discriminate coffees with significant differences in their aroma profile. For espresso coffee, the electronic nose was employed to discriminate four commercial blends with an array composed by four $SnO_2$ thin films sensors (Pardo et al., 2000). Coffee was sampled in three successive preparations: as beans, ground (powder), or liquid (the actual espresso). The measurements of liquid coffee did not give satisfactory results, and three out of four brands produced confusing results. The influence of humidity was deleterious because it imposes a big random noise on the measurements rather than a deterministic drift. For the measurements of the coffee beans, after an appropriate feature selection using PCA, an ANN with PCA scores as input gave 100% of correct classification. In turn, for ground coffee, the collected data presented a strong drift that hindered an easy classification. Drift is one of the most serious impairments suffered by an e-nose; it makes the use of flexible calibration methods, such as ANN, impractical (Ghasemi-Varnamkhasti et al., 2010). In Pardo et al. (2000), a PCA plot of the data showed that for every class, the drift could be approximated by a straight line. Therefore, they first subtracted, for each class separately, the projection of the data on the first principal component (PC) in order to compact each cluster, and then calculated the PCs again. Since the drift was ruled out with the removal of the first PC, it should be possible to use ANN for a more accurate segmentation, and then 87.5% of correct classification was achieved. A recent work evaluated six single varieties of arabica beans (Brazil, Ethiopia, Rio Minas, Guatemala, and Peru) and the certified Italian espresso blend using the Pico-1 electronic nose (Pardo and Sberveglieri, 2002) with five semiconductor $SnO_2$ based–thin film sensors. The obtained dataset was classified and correlated with panel test descriptors using a PCA combined with an MLP. Classification performance figures of over 90% and a good correlation were achieved with a hedonic index, but for individual quantitative descriptors, the results were not as reliable. In a subsequent work, the same data set for espresso coffee was classified using SVM and PCA for dimensionality reduction (Pardo and Sberveglieri, 2005). Pardo and Sberveglieri showed how the performance of SVM strongly depends on the technique for parameter selection, and that principal components carrying small variance have an impact on SVM performance. More recently, five different blends, the same data set described in Pardo and Sberveglieri (2002), were discriminated using an electronic nose and RVM with PCA for data analysis (Wang et al., 2009). Experimental results show that the RVM method is an effective technique for the classification of electronic nose data. Compared with SVM, the RVM can provide similar classification accuracy with dramatically fewer kernel functions. In addition, another advantage of the RVM method is that it has fewer parameter settings, in which case only one kernel parameter is needed.

Another possible application of an e-nose is to determine the forgery of coffee. The forgery could be made, for example, by mixing two different quality coffee brands: a mediocre product and a high-quality coffee type. Adding a small amount of low-quality grains to high-quality grains does not change the smell significantly, and therefore, is very difficult to discover the forgery. Very similar samples accentuate the problems of low sensitivity and instability of measurement associated with the change of environmental parameters, limiting the e-nose application. To overcome this problem, it was proposed using the differential signals of the two identical arrays of semiconductor sensors (Brudzewski et al., 2012). This solution differs significantly from the classical approaches to electronic noses, because two identical arrays of sensors are applied and only differential signals of both the arrays are processed in the pattern recognition system. In this way, the negative effect of the bias and baseline changes, resulting from the changing environmental conditions, are suppressed. In the mentioned work, both sensor arrays are composed of 12 MOS of Figaro series: 2xTGS2600-B00, 2xTGS2602-C00, TGS2610-C00, TGS2610-D00, TGS2611-C00, TGS2611-E00, 2xTGS2612-D00, and 2xTGS2620-C00 (Figaro, USA). The differential electronic nose in tandem with SVM obtained low classification errors (0.21%) to classify arabica and robusta blends. Using a classical e-nose with a single array of sensors, the average recognition error was equal to 2.95%, showing that the application of sensors working in a differential mode increased the sensitivity of the measurement system and made it less susceptible to a change of the environmental conditions, since these changes are compensated for in the differential signal.

The electronic nose could be a very useful technique to support sensory evaluation because of its ability to acquire qualitative, low-cost, real-time measures of volatile compounds (Michishita et al., 2010; Pardo and Sberveglieri, 2002). The cup tests are performed by tasters; thus, this way of conducting quality control is very subjective, depending on the skill of the taster. Moreover, not all the tasters are able to find every defect in a cup of coffee. This problem can sometimes cause a loss of money and time due to a lack of standardization of cupping tests. But it could be solved by making a classification of coffee grains based on a more reliable instrumental analysis to identify defects in cups as the tasters do, but based on patterns of training rising, in this way, the influence of external factors (Rodríguez et al., 2010). The e-nose αFOX4000 (Alpha MOS, France), equipped with 18 MOS sensors contained in three chambers, was employed to analyze espresso beverage from six different arabica coffee beans (Brazil, Ethiopia, Guatemala, Colombia, Indonesia, and Tanzania) with three roasting degrees (light, medium, and dark; Michishita et al., 2010). The retronasal aroma simulator effluent gas was submitted to sensory evaluation and to e-nose αFOX4000; the sensor resistances and four sensory descriptors (roast, sweet, soy sauce, and earthy) were correlated using an ANN. The high correlation obtained between sensory scores and sensor array data showed that the e-nose was a useful tool to predict the results of sensory evaluation for aroma because the sample volume and human work needed for analysis were less than that for sensory evaluation, and the replication of analysis was relatively easy. Another work shows the application of an electronic nose called A-NOSE in the cupping tests for the detection and classification of defects (Rodríguez et al., 2010). The A-NOSE was developed at the University of Pamplona (Colombia) and the device is composed of a matrix of eight metal oxide gas sensors manufactured by Figaro (TGS-813, TGS-842, TGS-823, and TGS-800; Figaro, USA) and FIS (SP-12A, SP-31, SP-AQ3, and ST-31; FIS, Japan). The analysis included the comparison of some types of Excelso coffee (ie, coffee of excellent export quality) and Pasillas coffee (ie, coffee with many defects, which do not comply with the export standards). The defects detected in coffee cupping are often caused by a defect in the coffee beans; these defects are identified visually, but the degree of impact depends on the percentage of defects found in a given sample. The sensitivity obtained by the gas sensors was good, and the selectivity shown by the equipment was adequate. In addition, data processing with PCA and neural networks was successful. A previous work showed that the pattern of the sensor responses generated by the electronic nose could be used for performing triangle tests in two similar brands of 100% arabica coffee (Benedetti et al., 2004). Among discriminative tests, the triangle test is the most widely used test in food industries to determine whether the difference between two products is significantly perceptible. In practice, three samples are presented—two are alike, one is different—and the panelist is asked to select the odd sample. These sensorial tests require a panel of several people and is generally very time consuming and labor intensive for routine quality control application (Dutcosky, 2013). For comparison of an electronic nose with the sensory evaluation of coffee by triangle test, the analyses were performed using the e-nose 3320 Lab Emission Analyzer (Applied Sensor, Sweden; Benedetti et al., 2004). The e-nose's sensor bank is composed of 23 different sensors of which 10 sensors are MOSFET, 12 Taguchi MOS-type sensors, and 1 humidity sensor. The MOSFET sensors are divided into two arrays of five sensors each, one operating at 140°C and the other at 170°C, while the MOS sensors are mounted in a separate chamber and kept between 400 and 500°C during the entire process. While considering data obtained by the e-nose, all the replicates of the two coffee samples are correctly classified (100% of the time) showing that the device can differentiate similar samples only from the gas headspace components. On the other hand, no significant difference was detected between the two coffee samples by the sensory panel. From this work, the analytical utility of the electronic nose is evident so it can be used for the evaluation of different types of coffee, similar to the classical triangle test evaluation technique, but with greater speed and better performance.

For instant coffee, the e-nose was also applied to aroma pattern recognition using a PEN2 equipment (Airsense Analytics, Germany) to clustering and classification of seven different brands of soluble coffee (Bona et al., 2011, 2012). It was possible to correctly classify all samples, and the clustering methodology showed a clear separation between the classes. The PEN2 application for coffee indicated that two sensors were not sensitive to the aromatic profile of the analyzed samples. Furthermore, for the remaining sensors, there is a high correlation between the signal allowing the separation in three related groups (Bona et al., 2012; Romani et al., 2012). A similar distribution was obtained when an electronic nose with the same sensors, brand, and model was used for wheat (Zhang et al., 2007). Moreover, when a very complex mixture is analyzed (eg, coffee), the sensor responses are strongly correlated (Falasconi et al., 2005). It is therefore possible to infer that the correlation pattern is a result of the partial specificity of each sensor and is not related to the type of sample analyzed. When using the PEN2, the tester can use only one sensor of each group, thus reducing the redundant information for the multivariate methodology applied for e-nose data analysis. The more "orthogonality" the sensors show, the higher the obtained discriminative performance of the array. With significantly enhanced "orthogonality," more information is available, and also when different sensing principles, that is, different transducers, are combined. To improve the "orthogonality," an electronic nose was used to discriminate different coffee brands and mixtures using a hybrid modular sensor system with MOS and MOSFET sensors combined with polymer-coated quartz microbalance (QMB), humidity, and temperature sensors (Ulmer et al., 1997). Values and units of the different sensor response signals are not comparable if different transducers are chosen. Hence, standardization is necessary; this was done by subtracting the average and dividing by the standard deviation of the sensor signals in all calibration measurements. Using plots of the PCA scores, a full separation of all coffee beans is not possible by using only one type of sensor. On the other hand, for the hybrid sensor system, all samples were clearly separated and the classification was possible.

## 4.5 CONCLUSION AND FUTURE CHALLENGES

In this chapter the major contributions of an electronic nose for coffee analysis was outlined. It is clear that the utility of using e-noses in an industrial context is high, and most works have in fact shown cooperation with industrial partners who made the samples and conditions of use available. The question remains: Why is there an absence of electronic noses in industrial processes? There are several reasons for the reluctance of the uptake of e-noses in an industrial context. First, their sensing ability is heavily affected by environmental factors: humidity and background noise, general drift caused by temperature, sensor variations, and sensor poisoning. To overcome these limitations, a set of actions should be developed. On the materials side, major focus must be given to the design and development of drift-free sensors that can be used reliably over a long period, and new material for achieving better selectivity. On the software side, researchers must apply the newly available linear and nonlinear algorithms based on the statistical learning theory to compensate for the core problems of stability and reliability of the sensors. Nonetheless, the future of the electronic nose seems promising because researchers throughout the world are increasing their attempts to develop innovative instrumental techniques and pattern recognition tools.

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## Chapter 5

# Bakery Products and Electronic Nose

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## 5.1 INTRODUCTION

Bakery products are a large family of popular foods, consumed by a wide range of people, due to their varied taste, relatively long shelf-life and low cost. They are characterized by recipes mainly based on wheat or other cereal flours, blended with other ingredients.

The process employed for their production can also vary a lot, providing different textures and sensory characteristics to the final products. The main common operations in the production of bakery products are ingredient metering, dough mixing, shape forming, baking, cooling, and packaging. Each of the aforementioned processing steps is of equal importance in determining the final characteristics of this food product category. There are many factors that contribute to the quality of bakery products; the latter is generally judged on the basis of their appearance characteristics, texture, color, taste, and aroma. Among them, aroma profile is one of the most important attributes, being an important indicator of product quality and conformity, and influencing consumer acceptance and preferences.

The odor perception of a product is caused by several different volatile compounds and their relative amounts in the headspace determine the perceived odor (Heiniö, 2006). Aroma analysis in baked products first involves the extraction of volatiles from the food matrix, followed by the separation, identification, and quantification of the most relevant ones. To extract the aroma compounds from baked goods, besides traditional solvent extraction and simultaneous distillation-extraction (SDE), headspace analysis approaches (dynamic, purge-and-trap, or static analysis) and sorptive extraction techniques, such as solid-phase microextraction (SPME), have been applied (Paraskevopoulu, 2009). The identification and quantitative determination of aroma volatiles of bakery products is generally performed by GC–MS, which allows testers to solve most chromatographic overlapping problems. GC-olfactometry analysis has also been used for bakery products (Rychlik and Grosch, 1996). Recently, proton-transfer-reaction-MS (PTR-MS), which is a simple, fast sampling, highly sensitive method, was successfully used to monitor volatile changes in cheese crackers during storage (Pozo-Bayón et al., 2009), in dough and bread, and volatiles produced from yeasts in a fermented food matrix (Makhoul et al., 2014). However, many of these analytical methods are time and labor intensive, expensive, require skilled personnel to operate the equipment and to interpret the analytical results.

Chemical analyses of flavor compounds in bakery products can be combined with the sensory analysis. In general, the sensory traits of bakery products are determined by descriptive analysis, which gives a comprehensive view of the most important perceived product attributes (Heiniö, 2006). The vocabulary used is characteristic of each specific product and the selected attributes of their sensory profiles describe the odor, appearance, flavor, and texture, of which the last three are considered the most important features for cereal products (Heiniö, 2006). Different basic tests are frequently used to compare products and to determine whether they are different from each other or not (Cayot, 2007). These sensory methods need a group of well-trained assessors and several established attributes in order to provide reliable results without being subjected to individual breakdown or variation of sensitivity; such requirements are time consuming and, in some cases, could cause serious problems to some industries and laboratories (Sinesio et al., 2000). In the particular case of bakery products, the complexity of the matrices and aroma profiles sometimes limits the discriminating ability and perception of panelists, or the volatiles' concentrations can be close to or below the judges' detection limit (Yang et al., 2013). Therefore, an instrument such as the electronic nose (e-nose), with recognized high sensitivity, can proficiently provide complementary data to those attained with human sensory panels, can often replace a panel of human experts, and can perform odor assessment on a continuous basis, with reduced time and costs as compared to the aforementioned analytical techniques.

E-noses have been successfully applied to food and beverages analysis for process monitoring, shelf-life

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investigation, freshness evaluation, authenticity assessment, flavor and aroma identification, and other quality control studies (Peris and Escuder-Golabert, 2009; Deisingh, 2010). However, there are few e-nose researches focused on the study of aroma profiles of bakery products, since most works have been addressed to the different methods for the isolation and identification of their volatile compounds (VOCs).

This chapter provides a description of the main factors influencing VOCs generation in bakery products and their composition, with a focus on the main e-nose applications on bakery and cereal products.

## 5.2 FACTORS AFFECTING THE AROMA OF BAKERY PRODUCTS

The development of aroma in bakery products is due to a very large number of VOCs, which are related to ingredient quality and ratios (formulation), as well as their interactions. The peculiar aromatic characteristics of ingredients undergo several changes during dough-processing steps, including hydration, kneading, fermentation, baking, and postbaking treatments (Fig. 5.1).

During processing, these compounds are generated by enzymatic activity, fermentation, lipid oxidation, and thermal reactions (Rehman and Awan, 2011).

A brief description of the effects of some factors on the formation and release of aroma compounds in bakery products and their impact on product quality is provided in the following sections.

### 5.2.1 Effect of Ingredients/Formulation

The VOCs' composition of bakery products' aroma varies according to the type of ingredients, recipes, and aroma precursors used in their formulations. Different cereal and legume flours play a significant role in the generation of distinctive aroma and flavor in both leavened and unleavened bakery products; on the contrary, the contribution of wheat flour to the final flavor in bread making has been estimated to be small (Cho and Peterson, 2010) and has been also related to flour extraction rate (Rehman and Awan, 2011). Other ingredients (such as yeast, salt, dairy products, fats, sugars, sweetening agents, eggs, emulsifiers, spices, fruits, and nuts) greatly contribute to the final organoleptic characteristics of the different bakery products. The ingredients of baked foods are the main aroma precursor reactants (ie, sugars, proteins, lipids, and water) and have a strong impact on the principal reactions occurring during product making, such as Maillard reaction (MR), caramelization, and lipid oxidation. Water absorption in the matrix affects also dough rheological characteristics, which in turn affects color, aroma, and flavor development.

The effect of fatty matter and eggs on lipid oxidation and VOCs' release during elaboration of sponge cake was recently studied (Maire et al., 2013). The authors reported that lipid oxidation takes place during mixing and beating of ingredients into the dough; the presence of active enzymes (ie, lipoxygenases) in the raw material (especially flour), together with air incorporation during beating, could induce early lipid oxidation (Maire et al., 2013). When making bread and other similar products, the intensity and duration of kneading is one of the most important steps for the generation of aroma precursors by enzymatic activity. In fact, the concentration of some aldehydes (such as hexenal) in the breadcrumb has been found to be proportional to the intensity of kneading (Cayot, 2007).

### 5.2.2 Effect of Fermentation

In the production of bakery products, the fermentation conditions can widely vary, depending on the type

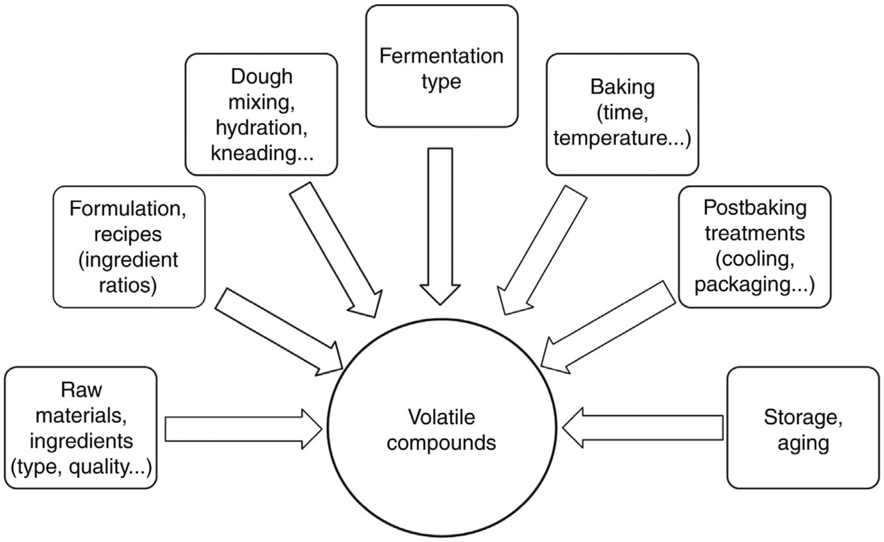
Fermentation type
Dough mixing, hydration, kneading...
Baking (time, temperature...)
Formulation, recipes (ingredient ratios)
Postbaking treatments (cooling, packaging...)
Raw materials, ingredients (type, quality...)
Volatile compounds
Storage, aging

FIGURE 5.1 Scheme of the main factors that contribute to the generation and modification of volatile compounds in bakery products.

of dough fermentation (by yeast and/or lactic bacteria or chemical/physical leavening agents) and process conditions. The microbial fermentation is the best in terms of aromatic characteristics developed in bakery products. The metabolic activities of yeast and bacteria initiate the fermentation process that produces organic acids, alcohols, esters, ketones, and aldehydes in fermented bakery products. Some of these chemical compounds further react to produce a variety of new aroma components during baking.

Sourdough fermentation is a traditional form of leavening used in the manufacturing of bread, cakes, and crackers. The sourdoughs are dominated by a complex microflora composed of yeasts and lactic acid bacteria (LAB), which play a significant role in the production of bread volatiles and nonvolatile flavor and aroma compounds (Rehman and Awan, 2011). These leavening agents produce gases (mainly $CO_2$), a wide range of aroma precursors that will be involved in cooking reactions, and other VOCs that will remain in the final baked products, contributing to their aromatic characteristics.

The composition of aroma VOCs is not influenced only by microbial composition, but is also affected by the interactive effects among types of bread-making process and ingredients used for the dough production (Rehman and Awan, 2011). Processing conditions (such as proofing time and temperature) and slackness of sourdough may affect the aroma volatiles. Long fermentation times can produce intense proteolysis and high amounts of free amino acids, which can act as precursors of Strecker aldehydes, mainly responsible for "malty" notes (Cayot, 2007). In general, low fermentation temperatures lead to longer fermentation times and produce a more appealing flavor (Rehman and Awan, 2011).

### 5.2.3 Effect of Baking

Baking can be defined as the process that transforms dough into a food with unique sensorial features (Purlis, 2010).

The greatest amount of aroma substances is formed during baking, due to thermal reactions such as MR between reducing sugars and amino acids, caramelization of sugars and lipid thermooxidation (Rehman and Awan, 2011; Cho and Peterson, 2010; Heiniö, 2006). MR and caramelization are catalyzed by a low–medium moisture level and the high temperature reached at the product surface during baking (Purlis, 2010).

Physicochemical changes occurring in dough during baking are very complex and take place following specific kinetics, depending on heating conditions and water activity. The main transformations induced by cooking are water evaporation, protein denaturation, starch gelatinization/destruction, browning and surface coloration (mostly due to MR), dough expansion, and thermal expansion of gas cells formed during mixing (Chevallier et al., 2000; Manley, 2001). In the baking process, the viscoelastic dough is transformed into a solid-like baked item. This process determines the final physical characteristics of products, including dimensions, weight, and moisture content. During the cooking process, a typical evolution of the aroma profile takes place. In the initial baking stages, heating produces an increase in the volatility of the distinctive aromas of raw food that derive from the ingredients used for their formulation. Thereafter, cooking involves the genesis and release of new VOCs produced by chemical reactions occurring in the food matrix. Finally, pyrolysis reactions (ie, MR and Strecker degradation) take place on the food surface with the formation of specific VOCs (Ward et al., 2002). Therefore, the aroma profile initially correlated to the raw matrix composition changes to a great extent, becoming very complex, in terms of type and quantity of aroma VOCs, at the end of the cooking process.

### 5.2.4 Effect of Aging

The deterioration of the aroma quality of bakery products during aging is due to the loss of some of the most important odor compounds and the formation of off-flavors also originated from lipid oxidation. After baking, some VOCs (such as pyrazines) disappear very rapidly by evaporation from the crust (Cayot, 2007). Aroma deterioration and changes may then result from volatilization of desired aroma and flavor components and development of undesired off-flavors (eg, from oxidative degradation), but compound migration between food matrix phases can also influence their stability (Heiniö, 2006; Yang et al., 2013).

The desirable alcohol smell of yeast is lost, the wheaty odor is reduced, and the leftover doughy or starchy aromas become unpleasant. The texture of the crumb becomes firmer and drier, whereas the texture of the crust becomes soft and leathery. In bakery products, the decrease in the flavor quality and a general staling reduces consumer acceptance, which is mainly caused by crumb changes rather than those resulting from microbial spoilage (Rehman and Awan, 2011).

### 5.2.5 Factors Affecting Volatiles' Release

It is well known that texture and microstructure of food systems impact the retention and release of aroma compounds during processing and storage (de Roos, 2003; Yang et al., 2012, 2013). Water loss, composition, size, and texture are the major factors that affect the aroma retention in bakery products (de Roos, 2006).

The release of volatile flavor compounds from baked products is controlled by the compounds' volatility in the product (thermodynamic factor) and the resistance to mass transfer from the product to air (kinetic factor); the latter is affected by the macro- and microstructure of the product. In complex solid and partly solid products, flavor compounds are entrapped in the hydrophilic or in the lipid phase across the matrix (de Roos, 2006). The food system can influence aroma release by physicochemical mechanisms, such as oil–water and water–air partition. With a relatively dry matrix, the rate and extent of hydration during eating will depend on the matrix composition, which may affect aroma release (Rehman and Awan, 2011).

In general, most organic flavor compounds are easily adsorbed and solubilized in lipids, depending on their lipophilic character. Proteins present in the matrix may influence the volatility of flavor compounds via weak Van der Waals interactions or by the formation of amides, esters, and salts. Polysaccharides can hinder the volatility of certain compounds, whereas other carbohydrates (such as monosaccharides and disaccharides) may cause a salting-out effect (Ampuero and Bosset, 2003).

## 5.3 COMPOSITION OF VOLATILE COMPOUNDS IN BAKERY PRODUCTS

Numerous researches have been carried out to study and depict bakery products' flavor using diverse instrumental analytical methods for compound identification. In general, the most important chemical functional groups involved in their aroma are aldehydes, alcohols, ketones, esters, acids, pyrazines, and pyrrolines, together with small amounts of hydrocarbons, furans, and lactones (Rehman and Awan, 2011). The composition of the volatile fraction can be greatly impacted by ingredients (such as the type and quality of vegetable oil), as observed in "taralli," a typical Italian snack food (Giarnetti et al., 2012; Caponio et al., 2013), in which more than 40 VOCs were detected. Grape marc extract, used for enrichment of functional biscuits, led to increased levels of some esters and aldehydes generated by fermentative activities, furans related to MR and lipid-derived compounds (Pasqualone et al., 2014); these VOCs resulted in a differentiation of the product sensory profile, but without defect detection. In biscuits formulated with green tea extract (GTE), hexanal was used as marker of lipid oxidation, showing that GTE was an effective inhibitor of hydroperoxide decomposition (Mildner-Szkudlarz et al., 2009). Partial replacement of wheat flour by soy protein isolate (SPI at 10% level) in cookies resulted in the highest yield (13.57%) of pyranones, the key odorants of cookies (Mohnsen et al., 2009).

During the fermentation process, acetaldehyde and diacetyl are produced together with alcohols and acetates (Hansen and Hansen, 1996). Bakery yeast starters produce characteristic VOCs in the dough [hydrocarbons (alkenes), carbonyls (esters, aldehydes, ketones), alcohols, carboxylic acids, some furan derivatives, and sulfur-containing compounds] (Rehman et al., 2006), which can affect the final product profile.

As the baking process starts, the rising temperature causes evaporation of highly volatile aroma compounds (acetaldehyde and diacetyl), so the presence of the latter is distinctive of the initial steps of baking process. VOCs produced during fermentation have also been detected in toasted bread, but only diacetyl has been found to significantly contribute to the bread smell (Rychlik and Grosch, 1996). MR products (such as pyrazines, pyrroles, furans, and sulfur-containing compounds) and lipid degradation products (such as alkanals, 2-alkenals, and 2,4-alkadienals) have been found in high-temperature processed cereal products (Parker et al., 2000). More than 540 VOCs have been detected in bread, but only a small portion actually contributed to its desirable aroma properties (Cho and Peterson, 2010). In fact, the volatile profile of partially baked bread is mainly constituted by Strecker aldehydes, 2,3-butanedione (diacetyl), 1-propanol, and 2-methylpropanol (Poinot et al., 2007). But diacetyl and 2,3-pentanedione (MR and sugar degradation products, respectively) are responsible for a buttery taste, related to rancidity (Caponio et al., 2013). The primary odorants that contribute to the flavor of wheat bread crust have been reported to be different from those of the bread crumb (Cho and Peterson, 2010); in particular, 2-acetyl-1-pyrroline has been suggested to be the key odorant of the crust, as well as of the cracker-like odor and the roasty odor at the beginning of wheat bread toasting (Rychlik and Grosch, 1996). The caramel-like smelling 4-hydroxy-2,5-dimethyl-3(2H)-furanone is mainly produced under medium browning conditions of wheat bread toasting, while concentrations of pyridines and pyrazines can change by a factor of 2–10 depending on the toasting degree (Rychlik and Grosch, 1996).

The aroma compounds change during aging of bakery products, favoring the formation and accumulation of off-flavors generated by lipid oxidation and/or spoilage. In crackers (Pozo-Bayón et al., 2009; Mandić et al., 2013) and oatcakes (with and without rosemary extract) (Cognat et al., 2014) that had been subjected to storage, the VOCs' profile was mainly characterized by aldehydes that derived from fatty acid auto-oxidation. In stored cookies supplemented with increasing SPI level, the appearance and accumulation of lipid-derived products was delayed, possibly as a consequence of the higher formation of MR products, since they have been shown to act as free radical scavengers (Mohnsen et al., 2009). Microbial spoilage (by bacteria, yeast, and fungi) and enzymatic spoilage (by lipoxygenase) can be differentiated from one another and from unspoiled bread by the generated VOCs' profile, before detecting visible marks of spoilage (Needham et al., 2005); among 59 identified VOCs, some of them were found to be produced by all spoilage types, while others were only generated by individual ones (such as pentanol produced only after 24 h spoilage by *Pichia anomala*).

Despite the large number of VOCs that have been detected in baked cereal products, not all of them have the same impact on the final aroma. The extent of their contribution will depend on their concentration as well as on their odorant power, which is related to their odor threshold (the minimum quantity of a compound that must be present to be detected by olfaction) (Cayot, 2007).

## 5.4 E-NOSE APPLICATIONS IN BAKERY AND CEREAL PRODUCTS

Several applications of the e-nose technique on various aspects of bakery products and food grains have been studied. Feast (2001) published an overview of potential applications of e-nose in cereals.

For bakery products and related raw materials, different types of e-nose systems, together with techniques for data processing and analysis, have been used for testing and discriminating aroma volatiles from different flours and ingredients utilized in formulation, processing operations (such as fermentation and baking), and storage conditions. A greater number of e-nose applications are focused on the rapid discrimination of VOCs for early detection of spoilage and fungal growth in cereal grains, and also in correlation with the presence of harmful contaminants, such as mycotoxins.

This chapter reviews some of these e-nose applications, which are also summarized in Table 5.1.

**TABLE 5.1** Applications of E-Nose to Bakery Products and Cereal Grains

| Purpose (Sample) | E-Nose Gas Sensor Array System[a] | References |
|---|---|---|
| **Ingredient Differentiation** | | |
| Bread | MOS | Sapirstein et al. (2012) |
| Bread | MOS | Torri et al. (2013) |
| Biscuits | MOS | Romani et al. (2006) |
| Infant cereal foods | MOS/CP | Li et al. (2010) |
| **Baking Stage Differentiation** | | |
| Bread baking aromas | MOS | Ponzoni et al. (2008) |
| Biscuits | MOS | Romani et al. (2012) |
| **Differentiation for Quality Aspects** | | |
| Toasted bread | MOSFET/MOS | Piazza et al. (2008) |
| Wheat flours | MOS | Adams et al. (2011) |
| **Aging** | | |
| Bread | $SnO_2$ | Botre and Gharpure (2006) |
| Wheat | MOS | Zhang et al. (2007) |
| Rice | MS-based e-nose | Sung et al. (2014) |
| **Microorganism Detection** | | |
| Bread | CP | Keshri et al. (2002) |
| Bread | CP | Needham et al. (2005) |
| Bakery product analogs | MS-based e-nose | Vinaixa et al. (2004) |
| Bakery product analogs | MS-based e-nose | Marín et al. (2007) |
| Paddy and maize | MOS | Wang et al. (2014) |
| **Mycotoxin Detection** | | |
| Barley | MOSFET/$SnO_2$ | Olsson et al. (2002) |
| Durum wheat | MOS | Abramson et al. (2005) |
| Maize | MOS | Gobbi et al. (2011) |
| Durum wheat | MOS | Lippolis et al. (2014) |
| **Lipid Oxidation** | | |
| Oatmeal product | MOS | Wessling et al. (2001) |

[a] *MOS, metal oxide semiconductors; MOSFET, metal oxide semiconductor field effect transistors; CP, conducting polymers; $SnO_2$, tin oxide semiconductors; and MS, mass spectrometry.*

### 5.4.1 Differentiation for Formulation and Ingredients

Some studies have been carried out to demonstrate the ability of e-nose to discriminate VOCs' composition and the final aroma of bakery products according to differences in one or more ingredients in their formula.

Sapirstein et al. (2012) reported that an e-nose having 12 metal oxide sensors (MOS) was able to differentiate between VOCs from refined and whole wheat bread samples, whose composition varied due to differences in flour, made with either white or red bran types. Moreover, Sapirstein and coworkers wanted to determine if the discrimination results could have been affected by the nature/presentation of samples (crust, crumb, or whole slice). Bread crust and crumb were better discriminated than whole bread types, probably due to the VOCs' blending. In another study, the sensory characteristics of six groups of bread, obtained with flours from different old versus modern organic common wheat varieties and treated with or without mycorrhizal factor, were compared by means of a sensory descriptive analysis, image analysis, and an e-nose composed of 10 MOS (Torri et al., 2013). The chemometric statistical analysis highlighted that e-nose was able to differentiate the groups of whole wheat bread samples as much as the sensory panel; moreover, e-nose examination was more efficient in predicting sensory variables of bread types than image analysis, except for the texture traits.

Romani et al. (2006) tried to discriminate the aroma profiles of differently formulated commercial biscuit types, produced by the same company, by using an e-nose equipped with 10 MOS sensors. Only in some cases e-nose responses were able to discriminate samples, especially when the latter were formulated with a high amount of butter, orange, or chocolate flavorings. Some raw ingredients were also analyzed but, in this case, the e-nose was unable to detect similarities between biscuits and their corresponding single ingredients, probably because of the great changes in the VOCs' composition due to baking, which increases the volatility of peculiar compounds deriving from raw ingredients and involves the genesis of new and more similar VOCs. However, the results reported in this paper referred to a limited number of data and could only be used as a preliminary proof of concept.

Li et al. (2010) examined the aroma quality of different commercial rice and barley infant cereals, by means of an e-nose with an array of MOS or conducting polymer (CP) sensors. E-nose responses were able to evaluate similarities and differences in aroma quality of infant cereal formulas compared with that of breast milk, which was used as a control and gold standard.

### 5.4.2 Baking Stage Differentiation

The rapid detection of a whole set of VOCs or key aromas during baking can be of crucial importance at an industrial level with respect to the final quality of bakery products. Ponzoni et al. (2008) used a low-cost custom-built e-nose system, based on a resistance to period converter readout system that is suitable to handle a wide range of resistance values, to detect five bread-baking synthetic key aromas. The aromas were distinguished on the basis of their chemical nature (acetaldehyde, diacetyl, pyridines, and pyrazines) and discriminated in relation to the baking-process stages.

Romani et al. (2012) studied the evolution of the flavor release of lab-made biscuits at different cooking times (0–12 min) by means of an e-nose equipped with 10 MOS sensors. The e-nose allowed the discrimination among raw, under-cooked, well-cooked, and over-cooked biscuits. Similar and complementary information was obtained considering both e-nose data and traditional physicochemical cooking indexes (moisture, color, texture). The obtained results showed the suitability of e-nose to monitor online changes in the biscuit's aroma and cooking level, thus allowing both the improvement of aroma quality of final products and the optimization of the technological parameters.

### 5.4.3 Differentiation for Quality Characteristics

E-nose has been combined with other analytical techniques in order to better detect and differentiate bakery and cereal products according to some quality characteristics, important for the final consumption or for the use as high-quality ingredients.

Consumer preferences of dry bakery products are mainly related to their texture and aroma release. These quality characteristics can interact differently depending on the matrix structure organization (de Roos, 2003). In a study by Piazza et al. (2008), texture characteristics of toasted sliced breads were correlated with the release of VOCs that develop after matrix crushing. In this work, the analysis of aroma VOCs was performed by an e-nose equipped with 23 different sensors [1 humidity sensor, 10 metal oxide semiconductor-field effect transistors (MOS-FET), and 12 MOS]. Combining some mechanical parameters (obtained by means of an acoustic–mechanical technique) with responses of selected sensors, Piazza and coworkers efficiently classified sliced bread samples, evidencing that the structural dissimilarities between them reflected the aroma release differences. It was therefore concluded that the efficiency of texture/aroma description was improved by combining these techniques.

In another study, Adams et al. (2011) used an e-nose with 6 MOS sensors to differentiate the aroma intensity and quality of flours obtained from diverse wheat varieties grown in the same or different locations. Moreover, e-nose results were compared with the contents of phenolic acids, flavonoids, and total pigments in the flours, which were analyzed by high-performance liquid chromatography

(HPLC). E-nose responses proved to be able to effectively discriminate the studied samples and were significantly and positively correlated with several phenolic acid contents.

### 5.4.4 Detection of Aroma Changes During Aging

Botre and Gharpure (2006) used a four tin-oxide sensors array and self-organized map (SOM)-based e-nose system for the evaluation of bread aroma changes (alcohol, $CO_2$, and other flavor compounds) due to staling, during 3 weeks of storage. The system developed was able to accurately predict the bread state as either fresh or stale. In the same research, the authors successfully classified three different bread brands.

E-nose was also used to detect the quality changes of different cereal products during storage. Zhang et al. (2007) employed an e-nose with 10 MOS sensors to evaluate 5 wheat samples stored for different periods of time. The wheat samples were stored in granaries at room temperature and between 50 and 60% RH for 5 years. Thanks to an optimization of the sensor array by multivariate analysis of variance and loading analysis, e-nose successfully discriminated the aged wheat samples according to the storage duration.

In a more recent work, Sung et al. (2014) used a mass spectrometry (MS)-based e-nose to screen and qualitatively evaluate rice samples stored at four different temperatures for 4 months. Rice was also tested for fat acidity and sensory characteristics. Flavor volatile profiles determined by e-nose changed according to the storage time and temperature, with a parallel increase in fat acidity and a decrease of sensory characteristics. The MS e-nose system was thus able to distinguish with excellent sensitivity and selectivity the modifications in rice quality related to volatile-producing metabolic activities, during storage at different conditions.

### 5.4.5 Detection of Off-Flavors From Microbial Spoilage, Mycotoxins Presence, and Lipid Oxidation

Microorganisms induce the release of undesirable flavors during storage and the formation of unwanted metabolites such as mycotoxins in cereal grains used for the formulation of cereal-based foods and bakery products. Fungal spoilage, even at early stages, generally coincides with an increase of $CO_2$, carbonyl compounds, and other VOCs that can be easily monitored by e-nose.

Early detection of qualitative changes in VOCs production in bread analogs contaminated by molds was investigated over a period of 72 h by using an e-nose system with a 14 CP sensor array (Keshri et al., 2002). Volatile production patterns were compared to those generated by hydrolytic enzyme activity, as well as to the fungal population increase. The e-nose system was able to detect early mold spoilage and to differentiate between uncontaminated and differently contaminated bread samples.

In a similar study, Needham et al. (2005) used a CP-based e-nose to detect and differentiate microbial spoilage, caused by inoculated bacteria, yeast, and fungi, in modeled bread after 48 h, before the occurrence of visible spoilage. E-nose was able to discriminate bread samples on the basis of VOCs' profiles from different microbial spoilage, enzymatic spoilage (caused by added lipoxygenase), and unspoiled bread.

A more sophisticated analytical approach (SPME coupled to an MS-based e-nose) was used by Vinaixa et al. (2004) for early detection of fungal growth in bakery product analogs and to accurately predict early fungal spoilage in bakery products inoculated with different mold species (Marín et al., 2007). The MS e-nose responses were highly and positively correlated with the ergosterol content, as index of fungal spoilage estimation. Moreover, e-nose recorded signals and ergosterol levels were used to build prediction models of bakery product spoilage in less than 7 days.

Moldy status of paddy and maize samples were measured by Wang et al. (2014) with an e-nose system with 8 MOS sensors, during 6 days of storage at room temperature. High environmental humidity was used to induce different moldy levels. The processed e-nose responses were able to clearly distinguish and discriminate samples. Moldy status predicting models were also developed.

Specific fungal volatile metabolites were identified as indicators of mycotoxins' content in cereals. In particular, Olsson et al. (2002) showed the possibility to predict ochratoxin A (OA) and deoxynivalenol (DON) levels in naturally contaminated barley samples using the VOCs detected and quantified by either GC–MS or e-nose with MOSFET and $SnO_2$ sensors. The barley samples were also analyzed for moisture content, fungal contamination, ergosterol content, and OA and DON levels. Using the VOCs detected and quantified by either GC–MS or e-nose, Olsson and coworkers were able to predict DON levels with a higher accuracy than OA levels, since different VOCs were either positively or negatively correlated with DON.

Abramson et al. (2005) monitored odor volatile evolution in durum wheat samples at different initial moisture content (16 and 20%), during 20 weeks storage, as an indicator of microbial infection, ergosterol, and micotoxin formation. Nine of the 12 e-nose MOS chemosensors were able to distinguish between VOCs, as well as to track their changes in wheat samples. Moreover, the responses of some specific chemosensors showed a good correlation with OA, citrinin, and ergosterol in the wheat sample at 20% initial moisture content.

An e-nose based on a 6 MOS sensors array has also been employed to diagnose fungal contamination in maize cultures inoculated in *Fusarium* species and to detect and predict fumonisin contents above and below the legal limits (Gobbi et al., 2011). E-nose was able to perform a rapid screening and a correct prediction of fumonisin levels in maize samples.

In another study by Lippolis et al. (2014), an e-nose based also on MOS sensors was successfully used to rapidly discriminate and classify large numbers of durum wheat samples with different DON contents (from <1000 to >2500 mg/kg), allowing to reduce the number of HPLC analysis. Moreover, an SPME/GC–MS method was developed to characterize the VOCs associated to the DON content.

The response of an e-nose can also be utilized as a fingerprint of off-flavor VOCs associated with lipid oxidation during storage, which can lead to organoleptic deterioration of some cereal products. In a study by Wessling et al. (2001), the oxidative stability of a commercial high-fat oatmeal product packed in four low-density polyethylene (LDPE) films (with diverse incorporated antioxidants into the polymers) was determined during storage by GC–MS and MOS-based e-nose. No significant changes in hexanal levels were observed, while the e-nose was able to detect variations in VOCs' profile (probably ascribable to early oxidation products) among samples and during storage time. Wessling and coworkers concluded that e-nose is a sensitive method for early detection of some oxidative variations in oatmeal during storage.

## 5.5 CONCLUSIONS

Most of the results of the studies reported in this chapter prove that e-nose can be very useful for evaluating different quality aspects of cereal and bakery products. In particular, e-nose has demonstrated to be a rapid and sensitive mean for controlling and monitoring some important processing steps of bakery products, for predicting and early detecting fungal spoilage, mycotoxin contamination and deterioration phenomena in raw materials and bakery products, also during storage. Therefore, among methods based on odor classification for determining the quality of cereals and bakery products, e-nose represents a useful alternative to replace and/or reduce the analysis for routine quality control, even though it does not provide a precise quantification and, in most cases, the obtained results need confirmation.

All the aforementioned results are promising and further studies are required to optimize the technology of chemosensors, with higher sensitivity and discriminatory power, in order to improve the recognition performance of e-nose and its potentiality and suitability for evaluating odor volatiles' characteristics in bakery products and related raw materials.

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## Chapter 6

# Electronic Noses for Monitoring the Quality of Fruit

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## 6.1 INTRODUCTION

Nowadays, expert and consumer panels are used widely to assess the quality of fruit. They can be considered human sensors to quantitatively measure the quality attributes of the product. The advantage of both expert and consumer panels is that they address the quality attributes of fruit and vegetables similarly to the ultimate consumer. However, even when obtained by a well-trained quantitative descriptive expert panel, scores are prone to large variability and may drift over time, the capacity of the panel is limited to typically 6–8 objects per session, the procedure is slow, and the cost is high.

Quality attributes can be objectively measured by both destructive and nondestructive techniques. Nondestructive techniques are often fast and have the particular advantage that the measurement procedure does not affect the characteristics of the fruit. The immediate benefit is that such techniques can be used for grading individual fruit and vegetables with respect to quality prior to sale. Because of the large biological variability of the quality attributes of fruit and vegetables, grading individual products is essential to meeting consumers' expectations. Color and size grading by visual inspection has been used for ages to remove products that would not meet the minimal requirements for quality and to simultaneously enhance uniformity. Over the years, this has been automated, and high-speed grading lines using sensors for external quality attributes, such as color, size, and appearance are now used widely by growers, cooperatives, and packing houses worldwide. The advent of nondestructive methods to measure internal quality attributes, such as texture properties or flavor, opened up exciting new marketing possibilities for horticultural products, provided, that the properties they measure correspond to their human analogues. Nondestructive techniques are also very useful for developing models of changes in quality attributes during postharvest storage, to optimize postharvest processes.

Among the different strategies for nondestructively assessing internal quality indicators in fruit, electronic noses have been receiving continuous attention. The pioneer studies, which date back from the late 1990s, established that electronic nose systems were very promising for nondestructively determining fruit quality for a number of reasons:

- They are based on inexpensive, nonspecific solid-state sensors, which are sensitive to the volatile compounds emitted both by climacteric and nonclimacteric fruit during ripening, shelf life, or cold storage. In particular, such sensors can be made very sensitive to ethylene (the ripening hormone in climacteric fruit, such as apples, peaches, bananas, etc.).
- Damage to fruit is often used as a criterion of quality (ie, rust fungi, formation of cork, bitter pit, insect damage, etc.). Diseases may result in telltale volatile fingerprints emitted by fruit, even at early stages when external damages are not yet visible. When properly trained, the electronic nose could help to discriminate healthy from diseased fruit in those early stages.
- The electronic nose is an instrument able to recognize, sort, and categorize volatile fingerprint patterns emitted by fruit. Rather than identifying and quantifying a restricted set of chemical compounds just like instrumental methods do, the electronic nose relies on pattern recognition performed on the fuzzy information generated by the interaction between the array of nonspecific gas sensors and the complex mixture of volatiles emitted by fruit. In that sense this instrument works more closely to the principles of a sensory panel than any other instrument does.
- Once an electronic nose has been trained, it does not necessarily require a skilled operator and can obtain the results in a few seconds. Additionally, in contrast with expert and consumer panels, such a system would never

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get tired of assessing fruit, which translates into a high-quality assessment throughout.

- Given the high flexibility that exists in the configuration of electronic noses (from desktop to portable and even hand-held configurations), the system may find application at any point of the production, logistics, and vending chain of fresh fruit. These potential applications consist of, but are not necessarily limited to, helping the farmer to decide the optimal time of harvest, sorting fruit at packing industries, continuously monitoring of fruit during cold storage and transport, monitoring fruit during shelf life, or helping consumers to pick fruit according to their preferences.

Despite the sustained efforts conducted for over 20 years now, the use of electronic nose technology for assessing fruit quality still happens at the laboratory level and for research purposes mostly. This chapter reviews how the electronic nose technology and applications for fruit monitoring have evolved in these 20 years.

## 6.2 ELECTRONIC NOSES FOR MONITORING THE RIPENESS STAGE OF FRUIT

The concept of electronic nose was coined in the early 1990s. A few years later Benady et al. (1995) and Simon et al. (1996) were the first to study the use of electronic noses for assessing the ripeness stage of fruit. Two years later, Maul et al. (1998) experimented with a commercially available Electronic Nose (e-NOSE 4000 from Neotronics Scientific Inc., USA), which consisted of 12 conductive polymers. This electronic nose proved useful at predicting in a nondestructive way, the number of days after harvest tomatoes would need exposure to ethylene for showing evident external symptoms of ripening. The predictive models were based on linear discriminant function analysis (DFA).

Later, Llobet et al. (1999) studied the effectiveness of different preprocessing techniques and neural pattern recognition engines for category discovering and classification of the ripeness stage of bananas and apples. In this paper, electronic noses comprised a limited number (four) of commercially available, resistive, metal oxide gas sensors. In general, the conductance of sensors was found to increase as the fruit ripened, which was associated to an increase in the number and the concentration of volatiles emitted by fruit as the ripening process progressed. No attempts for optimizing the type and number of sensors to integrate the array were made in these initial studies. Sample delivery was deliberately simple to assess the resilience of the electronic nose system to the presence of gas compounds in the background, the origin of which was exogenous to the process to be monitored (the evolution of fruit ripeness). That is why only two plastic vessels were used: The fruit to be monitored was placed in one of these two vessels (the sample vessel) and the other was kept empty (the reference vessel). During a measurement, a dynamic headspace sampling of the sample vessel was performed by pumping air from the sample vessel into the sensor chamber. The air pumped from the sample chamber into the sensor chamber was continuously replaced by air from the room. Immediately after the measurement phase, a cleaning phase started, which consisted of pumping air from the reference vessel into the sensor chamber. Similarly, the air pumped from the reference chamber into the sensor chamber was continuously replaced by air from the room. This allowed for regaining the baseline value of the sensors. The reasons for implementing this measurement procedure are twofold. The sample and reference chambers are identical and this enables to ensure that the electronic nose is responding to the volatiles emitted by fruit rather than to any residual smell of the plastic vessels. Exogenous volatile compounds present in the room equally influence both fruit responses and the baseline. In principle, the difference between the baseline and response signals would depend on the fruit only. In these pioneering studies, the pattern recognition (PARC) engines of the electronic noses employed for assessing fruit ripeness consisted of different neural network paradigms, such as the multilayer perceptron (MLP), the learning vector quantization (LVQ), or the fuzzy ARTMAP. All these algorithms implement a supervised learning strategy and, therefore, training vectors should be assigned a category (ie, a ripeness stage) before they can be actually used. Given the relatively low number of samples (and of measurements) available in these initial studies, the use of alternative and well-established instrumental techniques for categorizing ripeness could not be envisaged. Instead, unsupervised clustering methods, such as principal component analysis, self-organizing maps, or *c*-means clustering were explored for category discovering. These helped to classify samples in a few, relatively broad, ripeness stages that ranged from “green” to “overripe.” Even though the clustering methods considered work on different principles, these methods gave consistent results. In other words, the ripeness stage attributed to a given sample did not suffer from significant variations depending on the clustering method employed. Once any measurement in the measurement database had been tagged with a ripeness stage, the training and evaluation of the PARC of the electronic nose could be envisaged. Given the limited number of samples available, re-sampling techniques, such as the leave-one-out cross validation or the bootstrap method were implemented in an attempt to correctly estimate the success rate in the classification according to the ripeness stage.

With apples and bananas, Llobet and coworkers explored the use of the fuzzy ARTMAP, the LVQ, and the MLP neural networks for classifying their stage of ripeness. Similar accuracies above 90% were reached in the classification using fuzzy ARTMAP or the LVQ. It was found that these performances compared favorably with that achieved with back-propagation trained MLPs (slightly above 80%). The time needed for training the fuzzy ARTMAP was found to be typically more than an order of magnitude less than those for back-propagation MLP and LVQ. The generalization ability of the trained networks to the prediction of the state of ripeness of new, unknown samples was also investigated and it was found that the networks had a good performance, providing 90% accuracy in the classification of patterns belonging to previously trained categories. If a new category for which the network had not been trained occurred during testing, fuzzy ARTMAP and LVQ associated these patterns with classes that were the nearest to the actual state of ripeness and that were already known. Finally, the superior ability of fuzzy ARTMAPs to perform incremental learning without forgetting previously learned patterns was demonstrated in these applications, when it significantly outperformed LVQ and MLP, even in the presence of added noise.

However, in these initial studies, the sampling process was far too manual and rather slow. These inherent limitations made such systems difficult to adopt by the fruit industry or in real commercial applications. Additionally, further work to assess the long-term reliability of the system was needed because the effects of sensor drift on its accuracy were completely overlooked.

Young et al. (1999) compared the use of headspace gas chromatography coupled to mass spectrometry (headspace-GC/MS) and a commercially available electronic nose (the FOX 4000) from AlphaMOS (France) for sorting Royal Gala apples according to their ripeness stage. This system comprised 18 metal oxides (resistive gas sensors). According to the GC/MS study, 10 volatile compounds emitted from apples (acetates, alcohols, and aldehydes) could be identified, which showed evolution in their concentration levels as a function of the moment of harvest and number of storage days. Two data clustering models were built employing linear discriminant function analysis (DFA) on data gathered from the electronic nose and the GC/MS system, respectively. From the results, it was derived that the electronic nose slightly outperformed the GC/MS system and this was attributed to the higher sensitivity of metal oxide gas sensors to the volatile compounds released by fruit. Unfortunately, sample conditioning involved obtaining disks of the apple cortical tissue and, therefore, both the GC/MS and the electronic nose measurements implied the destructive testing of apples in this particular application.

Brezmes et al. (2000) employed a significantly higher number of samples in their analysis. Their experimental design included destructive testing of some fruit samples along the ripening process, which comprised estimating firmness, pH, and soluble solids employing a hand penetrometer, a desktop pH-meter, and a hand refractrometer, respectively. This strategy enabled them to follow the evolution of samples during the entire ripening process and also to sort them in three categories (green, ripe, and overripe). A manual static headspace sampling technique was implemented (Fig. 6.1), which included a step for allowing volatile compounds emitted from fruit to concentrate in the headspace of a large fruit vessel that could accommodate many fruit samples (ie, either peaches, pears, or apples). After the concentration phase, a fraction of the headspace was injected using an airtight chromatographic syringe into the sensor chamber. In a similar way to the previous studies, an arbitrary number of commercially available metal oxide gas sensors (ie, 12) were used to integrate the array. Fig. 6.2 shows how the sensor response increases with shelf-life days. Unlike in previous works, however, additional information, namely fruit weight and surface, were input to the back propagation-trained, MLP neural network engine of the electronic nose. This was done because it was considered that, besides the actual ripeness stage of fruit, variations in the total weight and surface of the fruit introduced in the concentration chamber would significantly affect the amount of volatiles present in its headspace after a fixed, always constant, time slot. Indeed, the inclusion of weight and surface information was used by the PARC engine as normalization factors that significantly improved the success rate in the discrimination of the ripeness stage of peaches, pears, and apples. Additionally, during the optimization of the PARC engine, a sensor pruning strategy was implemented. This consisted of using cross-validation

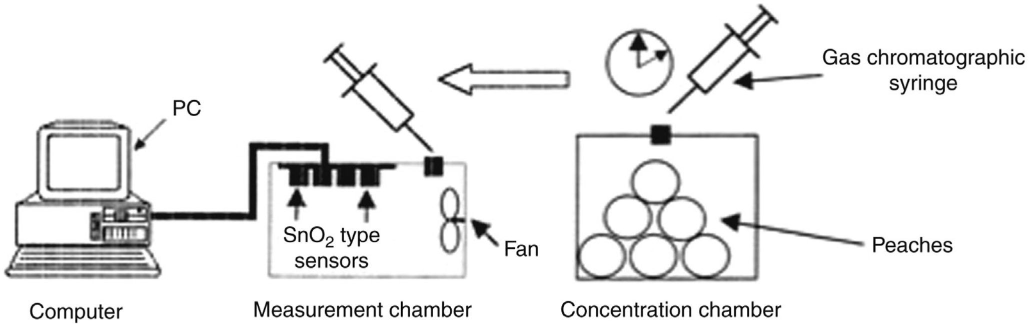

**FIGURE 6.1** A highly manual electronic nose to measure ripeness in climacteric fruit using a concentration chamber.

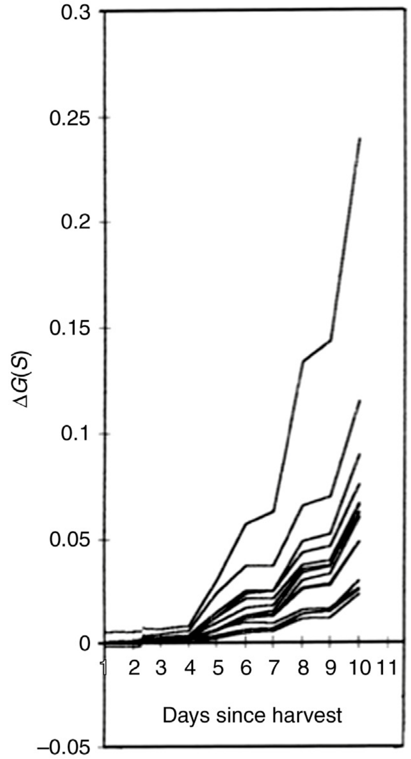

**FIGURE 6.2** Sensors increase monotonously as shelf-life fruit begins ripening after harvest.

techniques with a reduced set of samples for assessing whether removing sensors from the array would help improving the success rate in classification. It was found that using seven (peaches), two (pears), and one (apples) gas sensors together with weight and surface data would lead to the best results. Sensor pruning helps adapting the sensor array to the headspace generated by each specific fruit (eg, by removing redundant, irrelevant or noisy sensors), lowers the dimensionality of the PARC engine, and thus diminishes the risk of overfitting. A rather crude, three-category classification of fruit (green, ripe, and overripe) was attempted and while the success rate for peaches and pears approached 90%, it reached 73% only for apples, most probably because the sensors employed were not well adapted to the volatiles or the volatile concentrations found in the headspace of the latter.

Later, Brezmes et al. (2001) designed a 21-element metal oxide gas sensors array together with a fully automated dynamic headspace sampling and delivery system to assess the ripeness stage of Pink Lady apples along their shelf life. Fig. 6.3 details the general scheme of the system. Once more the gas sensors used were commercially available and destructive methods were employed to objectively identify the ripeness stage of apples (firmness, pH, and starch index). The main objective of the paper was to study whether the electronic nose would be able to accurately predict the results of the three destructive ripeness assessment methods. Indeed, by employing the responses of the sensor array to the headspace of apples and by building and cross-validating partial least squares models it was found a fair correlation between the responses of the electronic nose and the values of firmness and pH (Fig. 6.4, correlation coefficients higher than 0.93 and 0.84, respectively). However, a very important aspect of the paper is devoted to answer the following question: since metal oxide gas sensors are known to suffer from significant response drift, is the electronic nose really following the ripening process of fruit or simply responding to sensor drift? Ripening of fruit is a process that is largely related to time. It is generally assumed that the ripening process leads to a monotonic increase in the concentration of volatile compounds emitted by fruit and this should translate in a monotonic increase in sensor response (eg, increase in conductance change for resistive metal oxide gas sensors). Therefore, it is very important to closely monitor sensor drift to assess whether the results are achieved from the ripening process of the fruit alone or sensor drift is responsible for achieving meaningful results. This very important aspect had been completely overlooked in previous studies about fruit ripeness monitoring. Calibration measurements employing ethanol, a volatile compound to which the sensors were very responsive, which was not present in the headspace of the apples, were performed on a daily basis along the entire process implemented for

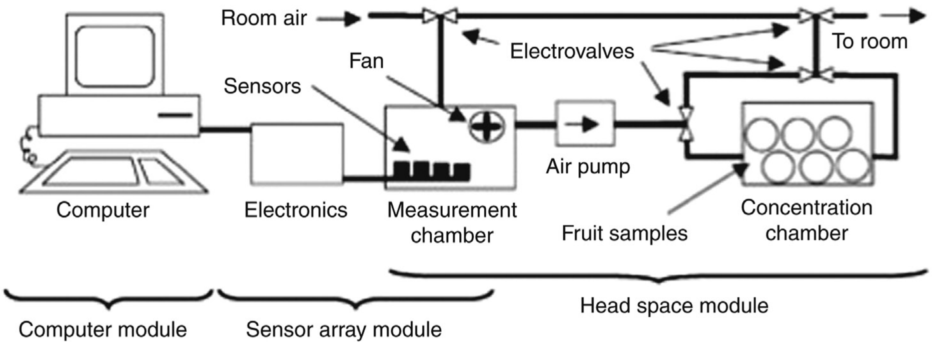

**FIGURE 6.3** General scheme of a highly automated electronic nose.

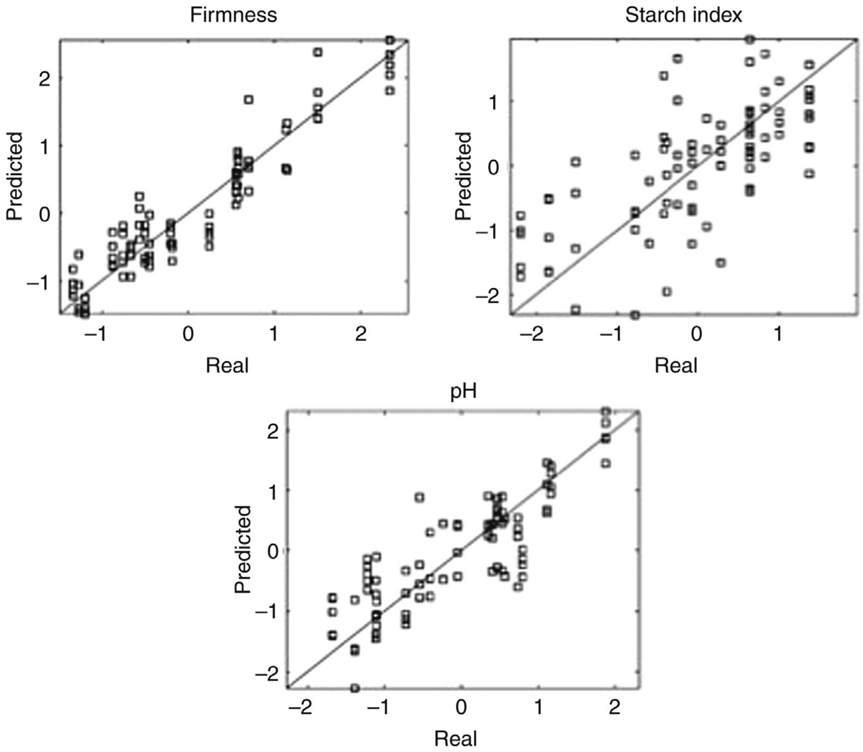

**FIGURE 6.4** **Electronic nose measurements correlate very well with firmness, somehow with acidity (pH) and they are almost uncorrelated to starch index values during shelf life.**

monitoring the evolution of ripeness of Pink Lady apples. Different clustering algorithms, such as principal component analysis (PCA) or fuzzy ARTMAP were used to identify whether similar clustering patterns occurred for calibration measurements and apple measurements, because that would clearly indicate the influence of sensor drift. It was found that 7 of the 21 sensors integrating the array were seriously affected by drift. When the response of these seven sensors affected by drift were used, the ethanol calibration measurement of a given day clustered together with apple measurements of that same day as it is shown in Fig. 6.5. Once these drifting sensors had been removed from the electronic nose array, a last check was performed, which consisted of using the ethanol calibration measurements to build and validate partial least squares models for predicting firmness and pH. The fact that these calibration responses resulted completely uncorrelated to firmness or pH clearly indicated that the 14 remaining sensors were not affected by significant response drift, at least during the timeframe of the experiment (~1 month). Finally, Brezmes et al. (2005) extended their study to other cultivars (ie, pears, nectarines, and peaches) showing that the electronic nose had good potential for predicting the optimal time of harvest, the ripeness stage of fruit along their shelf life, and also some quality parameters of fruit. Despite these good results, a few important open questions for improving the system were identified, such as devising a straightforward procedure for detecting and correcting sensor drift. Additionally, the calibration of the system for a given cultivar should take a few measurements only and be accurate, at least, for some consecutive campaigns. Finally, the measurement cycle should be faster in order to increase throughput.

Herrmann et al. (2002) made an original contribution to the field by developing sensors specifically designed for monitoring the postharvest ripeness stage of apples. When apple aldehydes are present, the mass of the coated quartz crystal microbalances (QCM) increases, which results in a measurable negative shift in the resonant frequency of the QCM. Herrmann and coworkers showed that their sensors could reversibly detect the target molecule with a 20 ppm limit of detection and some selectivity. They measured artificially generated concentrations of aldehydes diluted in nitrogen but, at that time, they did not apply their system to monitoring real apples. Later, Echeverría et al. (2004) reached similar results while studying the postharvest ripening of Fuji apples. In that particular case, they used also an array of QCM sensors but coated with different films (metalloporphyrins) than those used by Herrmann and coworkers. They could identify ethyl-2-methylbutanoate as a key volatile for following the postharvest ripening of Fuji apples.

Saevels et al. (2003) took a close look at the effects of the cultivar and the campaign on the ability of electronic nose models to predict the optimal time for harvesting fruit. So far, any study had considered assessing postharvest

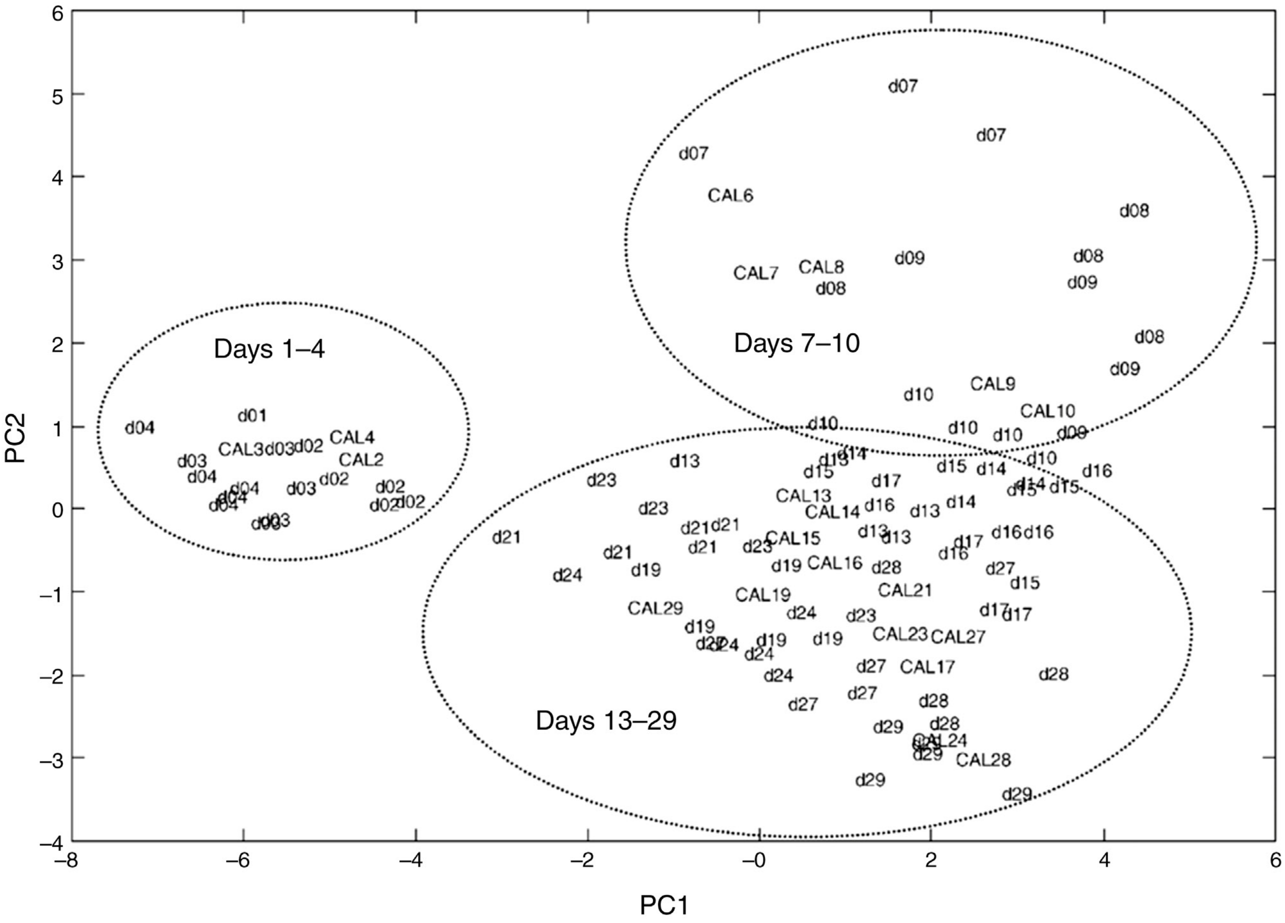

**FIGURE 6.5** **Calibration measurements *(labeled CAL)* follow a drift pattern that needs to be corrected.**

quality of single cultivars, for example, a given variety of apples, and of a single harvest year. The questions that Saevels and coworkers addressed were the following:

- Would a model for predicting the quality of a variety of apples perform similarly with a different variety of apples?
- Would a model for predicting the quality of a variety of apples, which was built employing samples from a given harvest year, perform similarly with the same variety of apples from a different harvest year?

Results clearly indicated that inter-cultivar variability prevented the electronic nose from performing well when it was asked to make predictions for a variety of apples based on the knowledge gained from the other variety of apples considered. Even when the electronic nose was trained with samples from the two apple varieties, the prediction ability of the system degraded in comparison to that of the system specifically trained for a single variety. Therefore, the answer to the first question above is no. This has serious implications for industrial use of the electronic nose system, because every year new varieties of apples are developed and marketed and results indicate that specific calibration would be needed for every single new variety to be monitored. Predictive models were also affected by a year effect. Indeed, it was impossible to accurately predict the maturity for harvest of apples if they had been harvested in a different year than the apples used for training the electronic nose. However, when samples from two consecutive harvest campaigns were used to train the electronic nose, the instrument was able to accurately predict the maturity for harvest of apples from both campaigns. Therefore, although the answer for the second question above is also no, from a practical point of view, the electronic nose shows good potential for estimating the quality of a given fruit variety along different harvest years provided that the system is trained on samples spanning through the whole set of harvest years.

## 6.3 ELECTRONIC NOSES FOR ASSESSING THE POSTHARVEST QUALITY OF FRUIT

Once harvested, fruits are subject to senescence in which different biochemical processes continuously change the original composition of the fruit until it becomes unmarketable. Despite all the care that is taken at handling, postharvest treatment remains a potential source of defect in fruits. Di Natale et al. (2001) studied the use of an electronic nose to detect postharvest defects in apples, namely, mealiness and the presence of skin cuts. The electronic nose comprised a seven-element sensor array of QCMs coated with metalloporphyrins, already described in Section 6.2. Apples with two degrees of mealiness (ripe and overripe) and with one and two cuts (cuts were produced with a tool that resulted in gauged and reproducible cuts) together with defect-free apples were measured by the QCM electronic nose. Given the fact that apple samples belonged to crisp categories (eg, having one, two, or no cuts), a partial least squares–discrimination analysis (PLS–DA) was implemented as a PARC engine. Since the total number of samples and measurements available was not very high, discriminant models were built and validated using the leave-one-out cross-validation approach. Under these conditions, the electronic nose was found to be able to detect mealiness and skin damage. The electronic nose was more sensitive to the presence of cuts than to the mealiness. This was attributed to the fact that increased mealiness did not change the nature of the volatile compounds found in the headspace of fruit. It only affected their concentration. On the other hand, skin cuts, apart from resulting in higher concentration of volatiles in the headspace due to direct flesh exposure, also trigger oxidation processes that produce new compounds.

Tan et al. (2005) addressed the detection of internal freeze damage in two different varieties of oranges employing an electronic nose and dynamic headspace sampling. The electronic nose consisted of a commercially available, 32-element, carbon-black polymer composite array (Cyranose 320; Cyrano Sciences, Pasadena, CA, USA). The PARC engine consisted of a canonical discriminant analysis for performing a binary classification of fruit (ie, sound or freeze-damaged). To estimate the success rate in classification, a leave-one-out cross-validation approach was implemented. Orange samples belonged to Cutter Valencia and Olinda Valencia. Freeze damage was produced artificially by storing fruit under freezing conditions for a controlled period of time. Freeze-damaged fruit had internal damage but externally they were undistinguishable from sound fruit. Specific prediction models worked similarly well for both cultivars and the success rate in the discrimination between freeze-damaged and sound oranges was about 72%.

Li et al. (2009) studied the use of an electronic nose to detect rot in mangoes. Rot in fruit is caused by bacteria and, more often, by fungi and results in skin lesions. The number and the size of skin lesions increase when rot worsens. The organoleptic properties of fruit degrade when affected by the disease. Li and coworkers employed a commercially available instrument the zNose (7100 Fast GC Analyzer, Electronic Sensor Technology, New Bury Park, CA, USA) to analyze the headspace of mangoes to detect the occurrence of rot. This equipment is a miniature, high-speed gas chromatograph, containing a short separation column, an uncoated (ie, broadly selective) surface acoustic wave (SAW) sensor as detector, and support electronics. Before the volatile compounds from the headspace of mangoes reach the detector, they are partially separated by the short column. The time a given component remains in the column is recorded as its retention time, which is supposed to be unique for each specific chemical. In fact, due to the short length of the separation column, many compounds may not be resolved and are coeluted to the detector. The derivative of the frequency signal is used as a quantitative measurement of the quantity of the chemical(s). An advantage of the zNose is that results are obtained much faster than in standard chromatography, which uses long separation columns. PLS and the variable importance for projection (VIP) were used to identify which peaks of the zNose could be used for predicting rot. In the end, a single peak could be used for assessing the presence of rot in mangoes with a 90% success rate in the classification. These results are encouraging for industrial use of this method in packinghouses for sorting fruit. However, in this study the diseased mangoes already showed the presence of visible lesions (rots) at side or stem ends and it would be worth investigating whether the method is sensitive enough to detect diseased fruit at very early stages, before the onset of skin lesions.

Li et al. (2010) employed an electronic nose comprising several conducting polymer sensors to assess fungal contamination in blueberries. In their experiment, ripe organic rabbiteye blueberries were hand-harvested and rinsed three times in distilled water to remove any residue before inoculation. Blueberries were punctured with a sterile needle on its stem side to create a slight wound to facilitate infection. Blueberries were infected with three types of fungi responsible for frequent diseases (ie, *Botrytis cinerea*, *Colletotrichum gloeosporioides*, and *Alternaria* sp.). Berries started to show signs of fungal growth on their surfaces on 6–10 days after inoculation and this is when electronic nose measurements started. The commercially available Cyranose 320 (Cyrano Sciences, Pasadena, CA, USA) was used to sense the headspace of the three different types of inoculated samples together with a fourth control group, consisting of healthy, ripe blueberries having undergone the same rinsing procedure and puncture as inoculated samples, but which were not inoculated. A Bayesian classifier was trained and cross-validated with the responses of the electronic nose, which reached a 90% of correct classification of blueberries in the four categories (healthy and three types of fungi). The main problem associated to this study was the fact that it was necessary to wait for the headspace of blueberries to build up for 12 h to improve the reproducibility and reliability of the results. Pallottino et al. (2012) performed a similar study on Valencia oranges inoculated with *Penicillium digitatum* and *Penicillium italicum*. The electronic nose comprised a seven-element sensor array of

QCMs coated with metalloporphyrins. Four types of samples were available, which corresponded to healthy oranges and three inoculated categories with increasing inoculation intensity. The PARC engine was based on cross-validated PLS–DA. When a four-category classification was attempted, the success rate in classification was rather poor (about 50%). When specific models were built for two-category classification (ie, healthy or inoculated), the success rate in classification ranged between 73 and 82% for lightly inoculated oranges and heavily inoculated oranges, respectively. Gruber et al. (2013) reached similar conclusions on *Penicillium digitatum* inoculated oranges, the headspace of which was monitored with a four-element conductive polymer array. These results indicate that the electronic nose could be applied in the identification of fungal strains in storage rooms, especially when the infection occurs in small percentages that are not easily identifiable by classic methodologies of inspection.

Demir et al. (2011) studied the use of an electronic nose to assess the occurrence of impacts during packaging of blueberries. A commercially available electronic nose, the EN 4000 (EEV Inc., Amsford, NJ, USA), equipped with 12 conducting polymer sensors was used. To simulate the intensity of impacts, blueberries harvested at the same ripeness state were split in three different groups. Those belonging to the control group were not impacted; the other two groups were dropped from 200 mm distance into a picking bucket having either a plastic lug (soft impact) or a steel plate (hard impact) at the bottom. These impacts did not result in skin rupture or leakage after treatment or during storage. The headspace of impacted and control blueberries was measured with the electronic nose starting from two days after impact and discriminant function analysis was implemented and cross-validated to classify blueberries according to impact intensity. At day 2, 80% of the samples were correctly classified and from day 10 onward, the success rate in classification raised to about 90%. According to these results, it seems possible to objectively classify the bruising intensity of the fruit using an electronic nose provided that the storage time (or shipping time) were known. The electronic nose sampling time was sufficiently rapid for permitting the analysis of subsamples for quality control but was too slow to enable real-time determination during packing.

## 6.4 MASS SPECTROMETRY–BASED ELECTRONIC NOSES AND SENSOR FUSION TECHNIQUES

A MS–based electronic nose consists of a gas chromatograph coupled to a mass spectrometer in which the separation column of the chromatograph has been either by-passed or kept heated to such a high temperature that all the compounds input at the injection port of the gas chromatograph are coeluted at the output. These compounds are then ionized and complex mass-charge spectra are obtained at the detector of the mass spectrometer. These spectra consist of multivariate information that may be processed by a wide spectrum of pattern recognition (PARC) methods, including those usually found in gas sensor–based e-noses. These instruments combine the reliability and stability of classical instrumental analysis methods and the speedy operation of standard electronic noses, since the time-consuming gas chromatography separation step is no longer used. From a conceptual point of view, MS and standard electronic noses share the same approach because they do not intend to identify and quantify every component in a complex mixture of volatiles. Saevels et al. (2004) employed such an instrument to analyze the quality of apples during shelf life. The performance of an MS electronic nose was compared against that of a seven-element QCM sensor array electronic nose. Both instruments were trained for predicting the number of days of shelf life for Jonagold apples that had been kept under cold storage. The collection of volatiles from the headspace of fruit was performed employing a solid-phase microextraction fiber. The volatiles trapped at the fiber were desorbed either at the sensor chamber of the QCM electronic nose or at the injection port of the MS instrument. The MS electronic nose gave slightly better results than the QCM system at predicting fruit firmness and estimating days of shelf life of apples. Similarly to standard electronic noses, MS-based instruments would need specific calibration for every different cultivars and these calibrations would be also sensitive to the year of harvest. The same group (Berna et al., 2004) studied cultivar differences and postharvest ripening of tomatoes employing the same experimental methods and instruments just described. They found that the MS electronic nose performed better than their QCM electronic nose at discriminating between the two tomato varieties studied (Tradiro and Clotilde). This discrimination was attributed to the higher contents of volatile components derived from the aliphatic amino acids metabolism and because of the lower terpenoids content of Tradiro in comparison to Clotilde tomatoes. Both instruments were able to follow the shelf life of tomatoes. However, the MS electronic nose was more accurate at predicting the initial days of shelf life.

The main differences between electronic nose configurations are based on the sensing technology behind them. Nevertheless, most systems share a common feature: They only use a single detection technology. That is why it comes as no surprise that several studies report on the use of more than one electronic nose for the same study, expecting to increase the suitability of the combination for the purpose envisaged.

Li et al. (2007) reported a very exhaustive study on the use of two different electronic noses to detect defects on apples. As mentioned before, each commercial electronic nose usually uses only one type of detection technology and using systems from different vendors can increase the chance of success for a given task. In their case, they used an E-nose and a zNose.

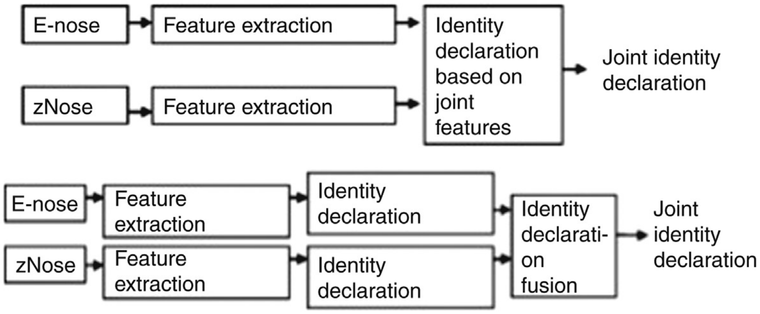

**FIGURE 6.6** Two different strategies to fuse data from two different electronic noses: feature extraction fusion and identity declaration fusion.

The E-nose (Smith Detection, Herts, UK) consists of 32 internal thin-film carbon-black polymer composite sensors, which can function at ambient air temperature. The zNose has been described before and comprises a capillary column and a SAW sensor.

In their study, Red "Delicious" apples were purchased from a local grocery store and were intentionally damaged by inducing a 10 mm deep cross-slice cut on the top. These damaged apples were exposed to room air for deterioration development. The measurements were conducted every other day from day 4 to 14 after the cut treatment. Other apples without the cut treatment were considered "healthy" apples. Apple samples were kept in room air for 6 h to reach the ambient air temperature before each test. Apples were maintained at room air temperature (20 ± 1°C) for 48 h between each measurement. A 2 L glass jar was used as a headspace gas concentration chamber, sealed by a plastic cap with a Teflon septum. The E-nose was used to sample volatile compounds emitted by the apples by inserting a 50 mm long snout needle into the 5 mm hole in the lid of the glass jar. The zNose was equipped with a 5 cm long sampling needle at the inlet, which was inserted into the concentration chamber for sampling. The sampling time was 10 s, during which the gas sample was released from the trap inside the system and carried over the column (DB-5) in a helium flow of 3 cm$^3$/min. Sampling was conducted at three different seasons: Mar., Jun., and Sep.

To fuse the data, two main strategies were proposed. Fig. 6.6 shows the two different approaches that differ in which stage the data fusion takes place:

1. Feature-level fusion: Features were first extracted by using PCA from each source of data (E-nose and zNose). These features were concatenated into a single feature vector, which in turn was used as input to an artificial neural network for classification.
2. Decision-level fusion: Data from each sensor individually was used to perform an identity declaration and the identity declarations provided by the individual sensors were combined using a Bayesian network decision-level fusion technique.

First, a PCA was used for feature extraction from raw data from each electronic nose. Then a probabilistic neural network (PNN) was designed for feature-based data fusion models. When fusing data, there are two issues that need to be addressed. The first one is the normalization of variables, which can easily be done with autoscaling, pareto scaling, or mean centering, depending on the nature of the data. This is a very well-studied problem and numerous solutions have been proposed. The second problem is trickier; it is called the "curse of dimensionality" problem. When training or fitting a mathematical model, it is always important to make that model as parsimonious as possible. To do so, the fewer parameters used to describe each measurement, the better. Of course, there is a lower limit since the training procedure has to be learned by the system. But, if too many variables are used, we can overtrain the system, which indeed produces a lack of generalization when new measurements (not used during training) are executed.

Li et al. (2007) as well as other authors (Di Natale et al., 2002; Boilot et al., 2003) used variable selection methods mostly based in genetic algorithms (GA). Different studies have benchmarked the performance of such approaches and the best option uses integer chromosomes, opposed to binary chromosomes, since they select fewer variables with a higher performance rate in validation (using samples or measures not used for training previously).

For example, in the study by Li et al. (2007), it was found that simply adding the E-nose and zNose raw data together in nonselective feature fusion worsened the classification results: the 32.5% error rate from the fused data was higher than from the E-nose (15%) and the zNose (23%) individually. In contrast, the GA-based method called "dynamic selective fusion," which jointly selected useful features from the E-nose and zNose, greatly improved the system performance with a zero classification error rate.

Comparing the feature-level and decision-level data fusion models, the dynamic selective feature-level data fusion achieved better performance (an average 1.5% error rate) than the decision-level data fusion (an 11% error rate). The decision-level fusion's performance depends on the performances of the two instruments. By using soft evidence from

BP classifiers, Bayesian network fusion can improve the individual sensors' performance by 2%. These results supported the claim that generally better accuracy is obtained by fusing information closer to the source.

## 6.5 OUTLOOK AND CONCLUSIONS

The success of any approach to assess the quality attributes of fruit and vegetables critically depends on how their measurement principle mimics the way humans perceive a particular property. In that sense, electronic noses are well positioned because their basic philosophy is to use bio-inspired strategies to assess the quality of fruit from the standpoint of the final consumer.

On the other hand, due to the intrinsic variability of fruit, techniques that measure nondestructively one piece at a time are far more useful because measuring globally a complete batch will not ensure the quality of each individual.

Therefore, if each fruit unit is to be measured separately, commercially viable techniques require very fast and highly automated measurements. In order to fulfill these requirements, electronic nose sensors need to increase sensitivity around three orders of magnitude, so that a preconcentration time for each sample is not needed. Newer generations of chemoresistive sensors based on nanotechnologies can be the key enabler technology to fulfill this condition, since their sensitivity has already reached that goal.

Other desirable improvements in sensors include reproducibility, faster response times, and immunity to ambient factors such as temperature and humidity.

Finally, software should be improved to minimize training and calibration efforts while maximizing generalization, both in application to different cultivars and different campaigns.

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## Chapter 7

# Possible Application of Electronic Nose Systems for Meat Safety: An Overview

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## 7.1 INTRODUCTION

Meat is a major source of protein in the human diet and is rich in minerals. It is one of the most widely consumed food product in the world. The Midwest has a huge market share in the meat industry economy of the United States. However, meat products perish extremely fast due to improper handling and storage. Over time, the quality of meat deteriorates due to the breakdown of the nutrients present in meat. This causes changes in color, texture, and odor. When the animal is alive, the microbial count is negligible or even absent (Chung, 1991). However, when the animal is slaughtered, microorganisms find their way into the carcass and lodge inside them until conditions become favorable for their growth. Intrinsic and extrinsic factors such as temperature, relative humidity, water activity, oxygen concentration, and exposure to light are some of the conditions, which favor the growth of microorganisms and subsequently cause unwanted physicochemical changes in meat. Consumption of spoiled meat products causes a variety of health risks to the human population. Also, microorganisms deteriorate meat quality and cause safety hazards resulting in significant economic losses. About 76 million food-borne illnesses occur each year in the USA, whereas the European Food Safety Authority reports that about 15% of the population of Europe become ill every year as a result of consuming unsafe food (Falasconi et al., 2012). Based on a USDA report, out of the total food-borne illnesses believed to occur, nearly 5 million cases of illnesses and 4000 deaths may be associated with meat and poultry products contaminated with pathogenic bacteria (Dewaal, 1996). The annual costs encountered in dealing with these food-borne illnesses in the United States ranges from $5 billion to $6 billion, with more than 66% of this cost alone being attributed to meat and poultry products (Dewaal, 1996). Hence, the government is looking for various avenues by which a safe, reliable, rapid, and economical meat quality inspection system could be developed and implemented.

Electronic noses (e-noses) which are intelligent quality sensors (IQS) simulate the operation of human olfactory sensory system is one of the approaches being looked into for “sniffing” out the volatile organic compounds (VOCs) emitted from food products due to the action of microorganisms. Depending on the VOCs detected, information on the quality of the food product may be obtained to an appreciable degree of confidence. Although cheap, reliable, and robust sensors have been developed for various industrial applications, the use of these sensors to specific food products have to be tested, perfected, and miniaturized. Like every human having his or her own characteristic odor being emitted, stored meat emits gases which, when analyzed, can give information about the status of the stored meat and, to an extent, the condition of the meat. Various conditions like the age, breed, diet, health, sex of the animal, and storage conditions influence the type of VOCs emitted. This adds to the complexity of developing an E-nose system that is reliable and consistent for meat applications. With the help of appropriate chemometric techniques, the smell patterns obtained from the sensing system can be processed to give an output, which gives information on the status of the food product.

For developing an effective electronic nose system to identify presence of spoilage or pathogenic microorganisms in meat, there has to be a strong understanding of the nature of VOCs being emitted by the meat product. This helps in designing the e-nose system based on the target VOC and developing a reliable validation system. There is quite a lot of information regarding the volatile gases emitted from the action of various spoilage bacteria in beef (Chung, 1991; Jackson et al., 1992; King et al., 1993; Intarapichet and Bailey, 1993). Various researchers have also highlighted the use of e-nose techniques to train and identify the volatile gases emitted from food substances (Schaller et al., 1998; Balasubramanian et al., 2012; Wilson, 2013). However, very few or little information is available on the use of e-nose to identify the volatiles emitted due to the action of pathogenic bacteria.

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For the development of an e-nose system, three major components need to be integrated together—a sampling system, a detection system, and a data processing system (Peris and Escuder-Gilabert, 2009). This chapter focuses on the development of E-nose systems for meat applications and reviews the work done in the past by a variety of researchers.

## 7.2 SAMPLING, IDENTIFICATION, AND ANALYSIS OF MEAT VOLATILES

Beef during storage emits various VOCs due to chemical changes occurring within as a result of the action of microorganisms and enzymes present. These VOCs can be qualitatively and quantitatively identified fairly accurately using analytical instruments such as a gas chromatograph (GC) coupled with a mass spectrometer (MS). Such information obtained can provide information on the condition of the stored beef and determine whether it has crossed the threshold for safe human consumption or not. Use of GC–MS requires sufficient expertise and development of a reliable analysis method is time consuming. Also, the instrument cost for operation and maintenance is high. Hence, alternate rapid sensing techniques, which are user friendly, accurate, sensitive, and reliable, would be a good choice for sensing volatiles from stored food products. This is where e-nose systems will be a popular choice.

### 7.2.1 Volatiles Sampling

Sampling of the VOCs is critical in obtaining desired results using the e-nose system. Proper sampling techniques also help in developing validation techniques that will help in training the developed e-nose system to target the desired "indicator" VOCs and boost the power of the nose. GC–MS is by far the most widely used tool for validating the performance of an e-nose system. Before being introduced into the GC, a suitable method has to be selected for sampling the VOCs. Static headspace and dynamic headspace samplings are the two ways in which the headspace samples are introduced into the GC. Static headspace sampling technique saves time and money and hence, is preferred. For complex substances like biological materials, which differ in molecular weight, polarity, and volatility, headspace sampling is the fastest and cleanest method for analyzing volatile compounds. This method also is easy to operate and can be used for a variety of sample matrices (Pawliszyn, 2002). Solid-phase microextraction (SPME) is one of the widely used techniques for volatile preconcentration and sampling. SPME sampling is rapid and does not require elaborate sample preparation.

Table 7.1 summarizes some of the characteristics of SPME filaments and the GC–MS columns used by various researchers for analyzing volatiles from meat headspace (Balasubramanian and Panigrahi, 2011). The time shown in Table 7.1 is strictly the time required for extraction and the time required for programming the GC–MS method. It can be seen that a sufficiently long time is required for analyzing the headspace, which could be minimized drastically by using appropriate intelligent sensors. For e-nose systems apart from dynamic and static sampling methods coupled with SPME, stir-bar sorptive extraction (SBSE), inside needle dynamic extraction (INDEX), and membrane introductory mass spectrometry (MIMS) are other methods used to introduce the VOC into the sensing chamber of the e-nose system (Peris and Escuder-Gilabert, 2009).

### 7.2.2 Volatile Compounds in Beef

Various researchers have conducted studies on the volatile compounds emitted from beef (Wick et al., 1967; Intarapichet and Bailey, 1993; Jackson et al., 1992; King et al., 1993). From their research, valuable information on the VOCs emitted from beef can be extracted. It is very difficult to pinpoint the exact composition of the volatiles in beef. This is because the amount and composition of the volatiles emitted is not uniform for every animal and depends largely on the age, sex, breed, environmental conditions, feed, and the anatomy of the animal studied (Chung, 1991). Raw meat does not have strong flavor but cooking of meat increases the flavor. Flavor development seems to be due to the thermal degradation of sugars, amino acids, nucleotides, and oxidation of fat. According to these authors, hydrocarbons do not contribute significantly to the flavor of meat, whereas aldehydes are considered to be potential flavor components in meat. Alcohols, carboxylic acids, esters, lactones, furans, pyridines, pyrazines, hydrogen sulfide, and other sulfide-containing compounds are some of the group of volatile compounds identified in raw, boiled, and roasted beef (Wick et al., 1967; Ramarathnam et al., 1991, 1993). Among the volatiles identified, hydrocarbons made up the largest proportion, followed by sulfur-containing compounds, aldehydes, ketones, and alcohols. Many sulfur compounds like dimethyl sulfide, methyl mercaptan, dimethyl disulfide, propylene sulfide, and hydrogen sulfide were also frequently noticed, and mentioned in literature (Intarapichet and Bailey, 1991; King et al., 1993; Stutz, 1978). Esters, which were also commonly noticed during beef spoilage, were attributed due to the presence of fatty acids and alcohols like ethanol and methanol. Methyl acetate and ethyl acetate were the two common esters noticed during beef spoilage. Aromatic amino acids and other amino acids were the source for the presence of aromatic compounds and a lot of nitrogen compounds identified in beef headspace. The previously mentioned compounds can be used as potential indicator compounds for identifying meat spoilage. Acetone, methyl ethyl ketone, dimethyl sulfide, and dimethyl disulfide were found to be suitable indicator compounds for meat spoilage (Stutz, 1978). Esters, ethanol, and aromatic compounds were not suitable indicator compounds, which were

**TABLE 7.1** Column and SPME Filament Characteristics Used by Various Researchers for Meat Analysis

| References | Sample | Sampling Procedure | SPME Filament Polarity | Column Used | Coating on Column | Column Polarity | Total Time of Analysis (min) |
|---|---|---|---|---|---|---|---|
| Ogihara et al. (2000) | *Salmonella*-infected ground beef | SPME<br>PDMS<br>CARB/PDMS<br>PDMS/DVB | Nonpolar<br>Bipolar<br>Polar | DB-624<br>30 m × 0.53 mm | 3 μm<br>94% dimethyl polysiloxane | Medium polarity | 92 (10[a] + 82[b]) |
| Intarapichet and Bailey (1993) | Ground beef | Tenax trap | NA | Fused silica (SE-54)<br>50 m × 0.3 mm | 5% phenyl-methyl silicone<br>0.52 μm | Nonpolar | |
| Jackson et al. (1992) | MAP beef<br>Strip loins | Tenax trap | NA | Silica capillary column (5 CB)<br>25 m × 0.32 mm | 5 μm | NA | |
| Arnold and Senter (1998) | Processed poultry | SPME<br>PDMS | Nonpolar | Capillary column (DB-1)<br>60 m × 0.25 mm | 0.25 μm<br>100% dimethyl polysiloxane | Nonpolar | 65 (30 + 35) |
| Senter et al. (2000) | Raw chicken | SPME<br>CARB/PDMS | Bipolar | 60 m × 0.32 mm<br>DB-1 column | 5 μm<br>100% dimethyl polysiloxane | Nonpolar | 30 (6 + 24) |
| Morita et al. (2003) | Fish | Distillation and solvent extraction | NA | DB-WAX<br>60 m × 0.25 mm | 0.25 μm | Polar | 184 (120 + 64) |
| Specht and Bates (1994) | Shallow fried beef | Tenax trap | NA | DB-1<br>60 m × 0.25 mm | 0.25 μm<br>100% dimethyl polysiloxane | Nonpolar | 54 (16 + 38) |
| Drumm and Spanier (1991) | Cooked beef | Steam distillation-extraction | NA | HP-5<br>50 m × 0.32 mm | 0.52 μm<br>5% phenyl and 95% dimethyl polysiloxane | Nonpolar | 417 (285 + 132) |
| Ruiz et al. (1998) | Dry-cured ham | SPME<br>PDMS | Nonpolar | 30 m × 0.25 mm<br>Restek | 1 μm<br>5% phenyl-95% dimethyl siloxane | Nonpolar | 105 (60 + 45) |
| Gianelli et al. (2002) | Dry-cured ham | SPME<br>PDMS<br>CARB/PDMS<br>DVB/CARB/PDMS | Nonpolar<br>Bipolar<br>Polar | DB-624<br>30 m × 0.25 (identification)<br>DB-624<br>60 m × 0.32 mm (quantification) | 94% dimethyl polysiloxane<br>1.4 μm<br>94% dimethyl polysiloxane<br>1.8 μm | Midpolar<br>Midpolar | 352 (300 + 52) |
| Insausti et al. (2002) | MAP beef strip loins | Purge and trap | NA | HP-5<br>50 m × 0.32 mm | 1.05 μm<br>5% phenyl and 95% dimethyl polysiloxane | Nonpolar | 69 (16+53) |
| Gorraiz et al. (2002) | Vacuum packed MAP cooked beef | Tenax trap | NA | HP-5<br>50 m × 0.32 mm | 1.05 μm<br>5% phenyl and 95% dimethyl polysiloxane | Nonpolar | 53 (10 + 43) |

NA, not applicable.
[a] *Indicates time of extraction of volatiles using SPME/Tenax/purge and trap/distillation.*
[b] *Indicates time of GC–MS oven program.*

also agreed upon by other researchers (Intarapichet and Bailey, 1991, 1993; King et al., 1993) apart from Stutz (1978).

King et al. (1993) identified and quantified volatiles present in raw beef. Two fractions of volatiles were extracted by supercritical carbon dioxide. One fraction consisted of the lipid-like condensable fraction and the other consisted of the noncondensable volatile fraction. The volatile fraction had a higher concentration of hydrocarbons (over 30% of total volatiles), especially the heavier ones (C17–C22). Diterpenoids, alkylbenzenes, alkylnaphthalenes, and also long-chain fatty acids were present only in the volatile sample. For the condensable lipid fraction, aldehydes were the major components, constituting over 40% of the total volatiles. Ketones were present in a lesser amount in the noncondensable volatile fraction when compared with the lipid fraction (condensable). The origin of 1,2-benzenedicarboxylic acid alkyl esters in the chromatogram was not certain. Because these compounds are often used as polymeric plasticizers, King et al. (1993) suspected its migration from the plastic wrapping to the food. An interesting fact reported by Jackson et al. (1992) was that a large number of volatile compounds originated from the packaging material. This is interesting because we have to now consider the packaging material too before analyzing the volatiles emitted from beef.

### 7.2.3 Volatiles Associated With the Growth of Microbial Flora

During meat storage, various spoilage and pathogenic microorganisms act upon these substrates and break down the food system resulting in its spoilage and subsequent bad odor. There is information from literature indicating that the various microorganisms have their own characteristic volatile compound being emitted if they are present (Chung, 1991; Intarapichet and Bailey, 1991). So, this can be used as a tool to identify the presence of that particular organism in beef without any cumbersome food analysis technique. Meat spoilage is accompanied by a buildup of free fatty acid content, change in the pH of the meat, a change in the oxidation–reduction potential of the meat, and a buildup of nitrogen compounds.

Intarapichet and Bailey (1991, 1993) and Stutz (1978) have studied the VOCs produced by spoilage bacteria from commercial ground beef. They identified 186 compounds from their study. The major microorganisms they identified in their study were *Lactobacillus, Brochothrix thermosphacta*, *Lactococcus*, Psuedomonads, *Moraxella*, and *Citrobacter* species (Table 7.2). *Pseudomonas* was the dominant species identified in the meat samples (Intarapichet and Bailey, 1991, 1993). Volatile sulfur compounds

**TABLE 7.2** Significant Volatile Compounds Associated with Some Selected Spoilage Microorganisms Identified by Intarapichet and Bailey (1991, 1993)

| Species | Significant Compounds | Comments |
|---|---|---|
| *Pseudomonas* | Methanethiol<br>Dimethylsulfide<br>Dimethyldisulfide<br>Dimethyltrisulfide<br>Methylthioacetate<br>Acetoin<br>Diacetyl | Chiefly sulfur compounds |
| *Brochothrix* | Ethylacetate<br>3-Methylbutyl 3-methyl butyrate<br>2-Methyl isopentanoate<br>3-Methyl-1-butanol acetate<br>2-Methylpropanol<br>2-Methylbutanol<br>3-Methylbutanol<br>2-Methylpropanal<br>2-Methylbutanal<br>3-Methylbutanal<br>Acetoin<br>Diacetyl | Mostly esters were produced |
| *Lactococcus* | 1-Butanol<br>1-Pentanol<br>2-Methylbutanol<br>3-Methylbutanol<br>2-Methylbutanal<br>3-Methylbutanal<br>Acetoin<br>Diacetyl | Mostly alcohols were produced |

(like methyl sulfide, dimethyl sulfide, dimethyl disulfide, and hydrogen sulfide) are some of the most commonly occurring compounds due to the action of spoilage microorganisms in meat products (Intarapichet and Bailey, 1993) (Tables 7.3 and 7.4).

Senecal et al. (2002) studied the volatile gases emitted by the growth of pathogenic and spoilage microorganisms on selected agar medium, simulating the conditions of model protein- and carbohydrate-based foods. For *Salmonella typhimurium*, a pathogenic bacterium causing most of the food poisonings, the specific VOCs obtained were primary alcohols, secondary alcohols, and methyl ketones. *Escherichia coli*, another important pathogen, produced an extremely prominent "indole" peak during their growth on tryptic soy yeast agar (TSYA), which was absent during the growth of the other pathogens and spoilage-producing organisms. This amino acid (indole) could be used as a marker compound for the detection of the presence of *E. coli* in food products. Roth et al. (1970) have reported the excretion of indole during early exponential growth phase of *Bacillus alvei* present in acid-hydrolyzed casein medium. This is an interesting finding, which could be investigated further for identifying indole as a potential "biomarker" in foods. The ability of SPME to detect volatiles at concentrations in the parts per billion levels could make this technique one of the important tools for developing rapid detectors (sensing tools) for food safety applications. The development of biosensors, e-nose technology, and the rapid development of the nanobiotechnology and nanoengineering sectors have addressed the need for developing standard validation tools to help in validating the sensor technologies developed. SPME preconcentration along with GC–MS fits this role perfectly.

## 7.3 ELECTRONIC NOSE SYSTEMS

The e-nose or an artificial olfactory system is analogous to a human olfactory system in operation and can be used as an analytical tool for the analysis of flavor compounds. However, the artificial olfactory system does not give any information about the compounds causing the aroma or about their identity. The aroma/odor is judged by analyzing the aroma or smell pattern. With the aid of appropriate pattern recognition techniques, like artificial neural networks (ANNs), the capability of the e-nose for recognizing similar aroma patterns or distinguishing it from other samples is enhanced (Siegmund and Pfannhauser, 1999). The principle of an artificial nose system or e-nose systems typically rely on an array of conducting polymer or metal oxide–based chemosensors, with partial specificity and capable of recognizing simple or complex odors. Effective hardware systems coupled with a reliable and consistent software system are the key components in the design of any e-nose system. Hardware components consist of an array of sensors, allied electronics, pumps, air conditioners, flow controllers, and software for controlling and monitoring the hardware components, data preprocessing, statistical analysis, and so forth, together comprising an artificial nose system. While combining these components together to develop an artificial nose system, reproducibility and repeatability of the system should be the watchwords. Once developed and trained, the e-nose system can detect or differentiate between samples within a matter of minutes. A comprehensive list of various techniques that can be employed for sensing the presence of microorganisms has been listed elsewhere (Arora et al., 2006). Almost all of the techniques listed have a detection time of more than 1 h and require skilled personnel to operate. An e-nose system, on the other hand, once trained and validated for a specific task, for example, detection of *Salmonella* in beef, does not require a trained operator to be used.

### 7.3.1 Types of Sensors

There are four major technologies currently used in the commercially available e-noses. Of these four technologies, metal oxide semiconductors (MOS) and metal oxide semiconductor field effect transistors (MOSFET) operate at high temperatures and are classified as hot sensors. These sensors are less sensitive to moisture with less carryover from one measurement to another. The other two technologies, conducting organic conductive polymers (CP) and piezoelectric crystals [bulk acoustic wave (BAW) and surface acoustic wave (SAW) sensors] operate at low temperatures and are categorized as cold sensors.

MOS and CP sensors are widely used in electronic nose applications for meat sensing. MOS sensors consist of a ceramic substance coated with a semiconducting metal oxide film and are heated to temperatures between 200 and 650°C. The semiconductor coating is either an *n*-type or a *p*-type semiconductor and the response of these sensors to a target gas is in the form of change in resistance (Galdikas et al., 2000). According to Schaller et al. (1998), MOS sensors are highly sensitive to ethanol and may be poisoned by compounds like sulfur and weak acids. This is of particular interest, because when the compounds in meat products are broken down, ethanol, sulfur-containing compounds, and organic acids, which result in esters and ethers, are some of the chief volatiles being released into the headspace of a meat package. This might adversely affect the sensor sensitivity and reproducibility. CP sensors exhibit a change of resistance when gas is adsorbed by the sensor (Balasubramanian et al., 2004). These sensors have a substrate over which an organic polymer–sensing element is deposited. This sensing element is deposited between two gold-plated electrodes by an electrochemical deposition method. When the volatile gases are absorbed on the surface of the conducting polymer, the voltage applied between the electrodes

**TABLE 7.3** Summary of Volatile Analytes Produced by Microorganisms Related to Meat Products Growing in Meat and Other Model Food Systems

| Product | SPME Sampling Mode | SPME Fiber | Chief Analytes Identified | References |
|---|---|---|---|---|
| *Salmonella enteritidis, E. coli, L. monocytogenes, Klebsiella pneumoniae, Pseudomonas aeruginosa* in TSB | Static headspace sampling | 100 µm PDMS | *P. aeruginosa* produced 3-methyl-1-butanol and phenethyl alcohol, *S. enteritidis* produced ethanol, 3-methyl-1-butanol, decanol, dodecanol, 1-propanol, tetradecanol, *E. coli* produced indole, ethanol, decanol, octanol and 1-propanol, *L. monocytogenes* produced ethanol, 3-methyl butanol, dodecanol, *K. pneumoniae* produced ethanol, 3-methyl-1-butanol, decanol, dodecanol, tetradecanol, 1-propanol | Arnold and Senter (1998) |
| *S. typhimurium* in tryptic soy yeast agar (TSYA) | Static headspace sampling | 50/30 µm DVB/CARB/PDMS | Primary alcohols (1-octanol, 1-decanol), secondary alcohols (2-undecanol, 2-tridecanol), methyl ketones (2-nonanone, 2-undecanone), 3-methyl-1-butanol | Senecal et al. (2002) |
| *S. typhimurium* in tryptone yeast NaCl super-broth | Static headspace sampling | 65 µm PDMS/DVB | Hydrogen sulfide, ethanol, carbon disulfide, dimethyl cyclopropane, 1-propanol | Siripatrawan (2008) |
| *S. typhimurium* in alfalfa sprouts—glass vial | Static headspace sampling | 75 µm CARB/PDMS | Dimethyl sulfide, carbon disulfide, heptane, acetic acid, ethyl acetate, methyl alcohol, ethyl benzene, 1-pentanol, 3-octanone, 3-octanol, 1-hepten-3-ol | Siripatrawan and Harte (2007) |
| *E. coli* O157:H7 in TSYA | Static headspace sampling | 50/30 µm DVB/CARB/PDMS | Indole, 1-decene | Senecal et al. (2002) |
| *E. coli* in tryptone yeast NaCl super-broth | Static headspace sampling | 65 µm PDMS/DVB | Dimethyl disulfide, ethanol, 2-nonanone, 2-heptanone, indole, pentyl cyclopropane | Siripatrawan (2008) |
| *P. aeruginosa* in TSYA | Static headspace sampling | 50/30 µm DVB/CARB/PDMS | 1-undecene, 2-nonanone, 2-octanol, 3,7-dimethyl (E)(*)-2,6,-octadiene-1-ol | Senecal et al. (2002) |
| *Penicillium* species on Petri dishes | Static headspace sampling | 100 µm PDMS<br>85 µm PA | Mono and sesqui-terpene hydrocarbons, geosmin, 2-methylisoborneol, isopentyl alcohol | Nilsson et al. (1996) |
| *Shigella sonnei* in TSA | Static headspace sampling | 50/30 µm DVB/CARB/PDMS | Methanethiol, dimethyl sulfide | Warren et al. (2007) |
| *E. coli, S. sonnei, S. typhimurium, K. pneumoniae, Bacillus cereus, L. monocytogenes, S. aureus* in TSB | Static headspace sampling | 100 µm PDMS | 1 Octanol, 1-decanol, dodecanol, 2 undecanone, 2-tridecenone, indole (*E. coli*), 1 octanol, 1-decanol, dodecanol, 2-nonanone, 1-undecene, 2-undecanone, 2-tridecanone (*S. sonnei*), 1 octanol, 1-decanol, dodecanol (*S. typhimurium*), 1 octanol, 1-decanol, dodecanol, 2-nonanone, 1-undecene, 2-undecanone, 2-tridecanone, 2-tridecenone (*K. pneumoniae*), 2-undecanone, dimethyl disulfide (*B. cereus*), 2-undecanone, 2-tridecenone, dimethyl trisulfide (*L. monocytogenes*), 2-tridecenone, dimethyl disulfide (*S. aureus*) | Elgaali et al. (2002) |
| *S. aureus, P. aeruginosa* in blood agar | Static headspace sampling | 50/30 µm DVB/CARB/PDMS | Isovaleric acid, 2-methyl butyric acid, isobutyric acid, 1-hydroxy 2-propanone, 1-hydroxy 2-butanone, butyric acid, 4-methylhexanoic acid (*S. aureus*), 2-amino-acetophenone, dimethyl disulfide, undecene, dimethyl sulfide (*P. aeruginosa*) | Preti et al. (2009) |

DVB, divinylbenzene; PA, polyacrylate; TSB, tryptic soy broth; PDMS, polydimethylsiloxane; TSA, tryptic soy agar; CARB, carboxen; and TSYA, tryptic soy yeast agar.

**TABLE 7.4** Detection Limits of Various Electronic-Based Biomolecular Techniques for Detecting Food-Borne Pathogens as Listed by Arora et al. (2006)

| S. No. | Detection Technique | Organism | Assay Time | Detection Limit |
|---|---|---|---|---|
| 1 | E-nose | *Pseudomonas aureofaciens* | 1–2 min | ppm to ppb |
| 2 | Immunosensors | *S. typhimurium* | 15 min | 20 cfu/mL |
| | | *S. aureus* | 150 s | $10^4$–$10^5$ cfu/mL |
| | | *E. coli* O157:H7 | ~7 min | $10^4$–$10^5$ cfu/mL |
| | | *L. monocytogenes* | ~20 min | $10^7$ cfu/mL |
| 3 | DNA biosensor | *E. coli* | | ~40 cfu/mL |
| 4 | Nanomaterials-based sensing | *E. coli* | ~30 min | $2.67 \times 10^{-2}$ cfu/mL |

ppm, parts per million; ppb, parts per billion.

changes and because of this, there is a change of resistance. This change of resistance is sensed and delivered as the output. These sensors are very sensitive to polar compounds. Although CP-based sensors show more drift (Annor-Frempong et al., 1998), these sensors have an advantage in that they can be used at ambient temperatures. But due to their low operating temperatures (<50°C), these sensors are very sensitive to moisture. Their lifetime is very short, which may be due to the oxidation of the sensor or due to contact resistances developed between the polymer and the electrodes because of the action of the volatile compounds. A common aspect of the MOS, CP, MOSFET, and quartz crystal microbalance sensors is the fact that these sensors are sensitive to polar compounds and hence, to water vapor (Haugen and Kvaal, 1998). During meat storage, the headspace above the meat samples is filled with a greater percentage of water vapor due to a relatively high water activity of the meat. This issue can be tackled either by adjusting the humidity of the carrier gas to be same as the humidity of the sample headspace or to adjust the humidity of the carrier gas to a maximum value. Another way of addressing this issue is to get the signals from samples of purified water adjusted to the same humidity as the meat headspace and then making a water background correction by simply subtracting the obtained water responses from the signal obtained from the real meat samples (Haugen and Kvaal, 1998). Overall, Haugen and Kvaal (1998) summed up their review by indicating that the sampling of the headspace is the most critical part of analysis. The other factors, which contribute toward obtaining reliable results using an e-nose are the sample headspace temperature, equilibration time, sample quantity, and sample surface area.

## 7.3.2 Analysis of Collected Data

Smell patterns obtained from the e-nose sensors are analyzed using various statistical and neural network tools. The data obtained from the e-nose experiments can be used either for prediction or for classification of unknown samples. When the data from the e-nose system is used to differentiate between meat samples being "safe" or "unsafe" for consumption or being of "good" or "bad" quality, classification techniques are used. Prediction techniques are more difficult especially since the output is to predict the presence of microorganism present (in log cgu/mL or log cfu/g) or predict a time period when the stored meat will spoil. With biological systems as unpredictable as meat, prediction data analysis methods need to be robust and tested rigorously to have a reliable e-nose system. Pattern recognition techniques like principal component analysis (PCA), partial least squares regression (PLSR), functional discriminant analysis (FDA), cluster analysis, fuzzy logic, or ANNs are widely used for recognizing the smell patterns in e-nose applications. Except ANN methods, the other pattern recognition routines just mentioned are based on a linear approach. Because gas sensor data typically follows a nonlinear pattern, nonlinear methods for data analysis would be a better approach to give robust and reliable results and increase the power of the e-nose system. In the case of biological materials like meat, where the variation between samples are large, a linear approach of data analysis would not be the appropriate method to obtain consistent and reliable results. In such cases, a combination of statistical methods with neural networks would be the right approach toward building a sound classification or prediction model.

### *7.3.2.1 Preprocessing Techniques*

The performance of an e-nose system is as good as its input data. If data/signals obtained from the sensors have a great deal of variance or interference (noise), then the classification and prediction algorithms will not perform properly and will give misleading results. Preprocessing the data helps in reducing this noise and boosts the differentiation capability of the e-nose. Caution should be employed not to overdo preprocessing because that will also distort the output obtained from the data processing algorithms. Various preprocessing techniques like binomial smoothing, averaging, normalization, and autoscaling have been used to preprocess the signals obtained (Pinheiro et al., 2002; Balasubramanian et al., 2004). Pinheiro et al. (2002) stated that the common preprocessing technique applied to the data prior to principal component analysis (PCA) was autoscaling. By autoscaling the individual data are mean-centered and divided by its standard deviation for rescaling with unit variance. This helps to prevent high sensor responses from dominating the analysis (Pinheiro et al., 2002). By using principal components as inputs to a neural network, there is a fairly high chance of reducing collinearity in the data (Haugen and Kvaal, 1998).

#### *7.3.2.2 Classification Techniques*

Annor-Frempong et al. (1998) studied the response of an e-nose to various intensities of "boar taint." They validated their results from the e-nose with that obtained from a sensory panel and a GC. While analyzing their data, they first used a canonical correlation approach to visualize the relationship between the GC measurements of two indicator odor compounds with those from the e-nose measurements or sensory panel measurements. Later, Annor-Frempong et al. (1998) employed a multivariate discriminant analysis method to classify the odors based on Fisher's linear discriminant function. This supervised pattern recognition routine was employed to classify the boar taint based on concentrations and responses. By their approach they obtained a 90% correct classification rate in the training data set for classification based on concentrations and about 53% for the testing data set. However, they obtained 100% correct classification based on responses for the training set data and 84.2% correct classification for the testing set data by using nonlinear neural network-based classification techniques. Recently, El Barbri et al. (2009) report a 100% classification accuracy while using an e-nose system for identifying sardines' freshness based on support vector machines (SVM), nonlinear data analysis technique for data analysis.

Boothe and Arnold (2002) performed PCA on the data obtained from a metal oxide–based e-nose. They reported that the PCA maps were able to differentiate (classify) the smell patterns obtained from different poultry meat samples (fresh and stored) and also between the samples stored at different temperatures. However, Siegmund and Pfannhauser (1999) reported PCA did not differentiate between cooked chicken meat samples, which were stored for 24–48 h at 4°C, and the samples, which were stored for less than 24 h. They therefore performed PCA analysis of the discriminant factors between the different data classes. This procedure minimized variances between the data sets and maximized the Euclidean distances between the data sets, resulting in a better separation. By this mathematical procedure, Siegmund and Pfannhauser (1999) achieved better differentiation between samples stored at different times. Arnold and Senter (1998) analyzed the VOCs emitting from poultry by different bacteria species using an e-nose and a GC–MS. They found the percentage area under the curve for a select number of predominant volatiles and reported them for each species of bacteria, which were inoculated in the poultry meat. The smell patterns, which were obtained from the 32 sensors in the e-nose, was reduced into two dimensions using multiple discriminant analysis technique. These patterns were then analyzed by cluster analysis (Sammon mapping) and then plotted as a map. An ANN was used to classify the smell print data. Finally, the data obtained from the e-nose and the GC–MS was used to compare the gases emitted from the different bacteria species. Their research showed promising results justifying the use of an e-nose for classifying food products, depending on their quality.

Ridgway et al. (1999) used a variety of statistical procedures like stepwise multiple linear regression, nonlinear partial least squares regression (NPLS), linear discriminant analysis (LDA), and analysis of variance (ANOVA) techniques to classify their data obtained from an e-nose to detect the infestation of mites in wheat. These statistical procedures were performed with and without variable reduction by PCA. From the sensor signals (in the form of change in resistance) obtained, they extracted five features: maximum deflection from the baseline (divergence), rate of increase in response on sampling (absorbance), rate of decrease in response on purging (desorbance), ratio of absorbance to desorbance (ratio), and area under the response curve (area). They obtained a classification accuracy of 83% for samples with and without mite infestation. Overall, they concluded that use of nonlinear techniques like ANNs could improve the discrimination of samples.

Neely et al. (2001) used an e-nose to distinguish between different types of meat. Their nose was equipped with semiconducting polymer film (14 in number) sensors. The data from the e-nose was analyzed by the linear discriminant analysis method. The underlying technique employed by this method for discrimination was to form linear functions of the data to maximize the ratio of the between-group sum of squares to the within-group sum of squares. These linear functions were orthogonal. After the linear functions were computed, the classification process was done by finding out the Euclidean distance of an observation from the group of centroids, projected onto the subspace defined by a subset of the linear functions (Neely et al., 2001). The observation was then assigned to the closest group. To evaluate the performance of this method, the "leave-one-out" cross-validation method of estimating the centroids was used. Their tests showed that their e-nose classified the meat types accurately. The ability of an e-nose consisting of six metal oxide sensors to classify between *E. coli* O157:H7 and non-*E. coli* O157:H7 strains was studied (Younts et al., 2002). Each sensor was sensitive to a particular element: alcohol, ammonia, air contaminants, hydrogen sulfide, relative humidity, and temperature. Younts et al. (2002) calculated the sensitivity and the specificity of their instrument to determine the performance of their nose in differentiating between the different strains of *E. coli*. The data from the sensors were fed into a back propagation neural network (BPNN). They concluded that the specificity and the sensitivity of the instrument varied depending on the output value used to classify the gas signatures. Overall, it was observed that the sensitivity increased with decreasing output value, while the specificity was observed to decrease.

Many times, if the sample size is small, then building a reliable prediction or classification model could be difficult. Similarly, even with adequate samples, it is critical to build a reliable and robust model that could perform satisfactorily in real-world conditions. Several techniques such as the leave-one-out (a variant of leave-*k*-out) method and the bootstrap method have been used for this purpose. The bootstrap procedure has been applied along with various other statistical procedures (Parke et al., 1999; Serneels and Van Espen, 2005) and ANNs (Gismondi et al., 2002) to analyze environmental, biological, and biomedical problems. Schaffner (1994) utilized the bootstrap technique to simulate multiple growth rate measurements of *Listeria monocytogenes* and *Yersinia enterocolitica* from single set of experiments. The bootstrap technique has been successfully applied along with the PLS method to identify 10 types of microorganisms (Serneels and Van Espen, 2005). They obtained a misclassification percentage of 3.5% using this method.

A single bootstrap sample is created by randomly drawing "*n*" observations with replacement from the original sample set. The classification developed on each bootstrap sample was validated against the original sample (data set) and the associated estimates of error of prediction were determined. For "*N*" bootstrap samples, the average estimates of error of prediction was further calculated. As this error of prediction is reported to be biased, the following method, as proposed by Efron and Tibshirani (1993) was used to determine the refined bootstrap estimator (error).

1. First, use the original sample as both the training (O) and validation set (O*). Let the error rate for this test be Err (O, O*).
2. Next, compute the error rate for both training (B) and validation (B*). Let this error rate be denoted by Err (B, B*).
3. Finally, use the bootstrap sample as the training set and the original sample as the validation set. Compute the error. Let this error be Err (B, O*).
4. The bias or optimism of the simple bootstrap analysis is now defined as the difference between Err (B, O*) and Err (B, B*) averaged over the "*N*" bootstrap samples. The refined bootstrap estimator is now given by the optimism added to Err (O, O*).

### *7.3.2.3 Prediction Techniques*

Gardner et al. (1998) described the prediction technique employed for predicting the type of bacteria growth phase from the data obtained from an e-nose. They extracted nine features from the signals obtained from each sensor in the e-nose, and developed BPNN prediction models. They also studied the effect of unnormalized and normalized (standard normalization, vector array normalization, and autoscaling) sensor responses on the models developed. Their model could predict the growth phase of the bacteria with an accuracy of 81%. While predicting the type of bacteria, their model gave a prediction accuracy of 100% in predicting the presence of *Staphylococcus aureus*. The presence of *E. coli* could be predicted with an accuracy of 92.2 %. Out of the nine features they extracted from the sensor response, the model which worked the best giving an accuracy of around 96% was obtained using the feature "minimum output," which determines the minimum sensor response in volts corresponding to the reference air cycle/sample inlet cycle combined with standard normalization of this feature (all data corresponding to the "minimum output" feature were normalized so that they lie in between 0 and +1).

Winquist et al. (1998) used the BPNN technique to analyze their data obtained and make predictions regarding the quality of selected food materials stored over a given period of time. They found that the prediction accuracy improved when the storage time increased. It was reasoned that the emission of more gases with increased storage time caused the prediction to improve. Blixt and Borch (1999) followed a multivariate regression analysis approach (PLS) to develop mathematical models to predict the degree of spoilage in vacuum-packaged beef. They used information related to the sensorial traits (like acidic, sulfurous, and spoilage odors) and the sensor signals to obtain the weighted regression coefficients and the $r^2$ values from the PLSR analysis. The best prediction model they developed with the data collected had an $r^2$ value of 0.94. Qu et al. (2001) describe in detail how they performed a PCA of the data they obtained from an e-nose after obtaining samples from swine barns. They identified three principal components and used these components as inputs for the ANN. They used an adaptive logic network (ALN) neural network approach in their research. The performance of the developed ALN was quantified by calculating the parameters' root of mean square error (RMSE) and mean absolute percentage error (MAPE).

They concluded that their ALN was well trained and could predict the odor concentration with less than 20% mean absolute percentage error. Lou and Nakai (2001) reported a detailed study about the use of an ANN-based model for predicting the bacterial growth phase in modified atmosphere packaged cooked meat products. They used both response surface methodology (RSM) and a back propagation neural network (BPNN) approach to build models for predicting the maximum specific growth rate and the lag phase of *Lactobacillus sake*. It was noticed that the BPNN-based model showed a greater accuracy than that of RSM. For developing the neural network model, the data obtained was preprocessed by normalization, that is, the data was converted between (0, 1) for sigmoidal transformation or between (−1, 1) for hyperbolic transformation. However, Lou and Nakai (2001) employed a sigmoidal transformation with a slight offset, thus transforming the data between (0.1, 0.9). The parameters used for comparing the two models were the root mean squares error (provides information of how consistent the model will be in the long run), the average absolute percentage error (a nondimensional quantity which provides a basis for quantitative comparison among several attempted models), the average absolute error (same function as that of the average absolute percentage error but is used when the absolute of the target value is small), and the determination coefficient ($R^2$). In addition to these parameters, the neural network model was assessed for its sensitivity by computing the following criterion: the variable sensitivity error (indicates the performance of the developed network if that variable is unavailable), the variable sensitivity ratio (the ratio of the variable sensitivity error and the error of the network when all the variables are available), and the ANN geometry (relates to the number of layers and the number of neurons which make up the layers). Overall, they concluded their study with a series of plots indicating that the BPNN model was more accurate in prediction than the response surface methodology (RSM) model. The higher accuracy observed in the ANN model was attributed to its ability to take into account the nonlinear characteristics of the data.

Table 7.5 gives an overall view of the various prediction and classification techniques used by researchers in analyzing data obtained from the e-nose system to analyze the quality/safety of meat products. Prediction and classification accuracies as high as >90% can be obtained using multivariate or ANN techniques. Combining sensor data from different types of e-nose systems, the "sensor fusion" approach (Balasubramanian et al., 2012) and using higher-order statistical techniques like independent component analysis (ICA) (Balasubramanian et al., 2008) are some approaches that have been used in the recent past to boost the discriminatory power of the e-nose system to identify the presence of *S. typhimurium* in stored beef strip loins.

## 7.4 CHALLENGES AND FUTURE DIRECTION

Electronic noses have been used in a variety of applications including meat quality and safety applications. The direction of electronic nose systems is toward miniaturization, improved repeatability and reproducibility, and the use of smart nanoscale materials for selectivity and improved sensitivity. Recent research suggests that the electronic nose systems combined with robust chemometrics could provide a greater degree of reliability on meat classification comparable to those obtained using traditional time-consuming microbial analytical methods. However, numerous challenges have to be addressed to make these systems reliable in real-world conditions. One of challenges is to select a robust type of sensor with a high specificity toward the compounds of interest in a particular application. The compounds of interest are generally a small part of a complex background including water vapor and carbon dioxide. This can be compared literally to "finding a needle in a haystack." The background volatiles interfere and reduce the sensing ability of sensors. Also, loss of selectivity and sensitivity of these sensors to the compounds of interest over time is a major challenge. One way to overcome these challenges is to move toward developing low-cost disposable sensors. Advancements in nanotechnology and sensory fabrication could definitely be helpful in taking the electronic nose technology to the next level. The other challenge is to develop a robust calibration model from a relatively small number of samples for meat quality and safety applications. This is further complicated by fact that native microflora of meat is dependent on the explicit conditions like storage temperature, relative humidity, and the handling of the meat. Further, implicit meat conditions like age and the sex and breed of the animal also contribute to the variations in the composition of headspace volatiles. Consequently, precision of the statistical and neural network classification and prediction models developed could be compromised.

Much progress is still needed in order to use the electronic nose technology as a reliable tool in real-world applications. Presently, the application of electronic nose technology for meat quality and safety is only limited to feasibility studies validated using only a few number of samples. On the other hand, it is very expensive to conduct a long-term study with a large number of samples to evaluate the technology in terms of repeatability and reproducibility. Numerous research reports have been published so far to demonstrate the theoretical and practical applications of electronic nose technology to predict meat quality and safety. With the meat industry and government cooperation and support, it is time for the researchers to fine-tune the technologies for practical and specific applications. This will require cooperative efforts between academics, government, and the meat industry to ensure a safe and quality meat supply.

**TABLE 7.5** Data Processing Techniques Used by Various Researchers for Meat-Sensing-Based E-Nose Systems

| Target | Sensor Type | Features Used in Model | Model Type | Data Analysis Technique Used | Results Obtained (Best) | References |
|---|---|---|---|---|---|---|
| Sardines freshness | Metal oxide | Change in conductance, dynamic slope of conductance | Classification using PCA, microbiological results | Support vector machines (SVM) (nonlinear) | 100% classification accuracy | El Barbri et al. (2009) |
| | | | Prediction using PCA | Partial least square (PLS) (nonlinear) | Correlation coefficient (*R*) between 0.90 and 0.91 | |
| Presence of formaldehyde in octopus samples | Metal oxide | Sensor resistance in air, sensor response over time, and sensor desorption rate | Classification | Discriminant function analysis (DFA) | 93.1% classification accuracy | Zhang et al. (2009) |
| Minced pork freshness | Metal oxide | Sensor resistances | Classification | LDA | 100% accuracy in recognizing meat from same supplier | Musatov et al. (2010) |
| Minced beef stored aerobically and under modified atmospheric packaging | Quartz crystal microbalance | PCA | Classification | SVM | 81% accuracy | Papadopoulou et al. (2011) |
| Beef freshness during storage | Metal oxide | Sensor resistances selected by LDA, stepwise-LDA, PCA, and Mahalanobis distance (MD) | Prediction | BPNN and generalized regression neural network (GRNN) | GRNN performed the best with LDA as the feature extraction method for predicting storage time, micropopulation, and sensory scores | Hong et al. (2012) |
| Predicting bacterial counts in chilled pork | Metal oxide | Sensor resistances between 6 and 50 s | Prediction | PLS-SVM | $R = 0.88$ | Wang et al. (2012) |
| Adulteration of minced mutton by pork | Metal oxide | Sensor resistances selected by stepwise- linear discriminant analysis (LDA), PCA, loading analysis | Classification | Canonical discriminant analysis (CDA) and Bayes discriminant analysis (BDA) | 92.44% by using CDA and features selected by stepwise-LDA | Tian et al. (2013) |
| | | | Prediction using features extracted by stepwise-LDA | PLS, MLR (multiple linear regression), BPNN | $R = 0.976$ by BPNN | |
| Total volatile basic nitrogen as an indicator of pork freshness | Metal oxide | Maximum resistance value of each sensor | Prediction | BPNN | $R = 0.6495$ by BPNN | Huang et al. (2014) |

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## Chapter 8

# Multivariate Approaches to Electronic Nose and PTR–TOF–MS Technologies in Agro-Food Products

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## 8.1 INTRODUCTION

The appearance of agricultural products is a crucial benchmark that ultimately affects final consumer choice (Kays, 1991). The variables contributing to appearance are the size, shape, form, color, freshness condition, and absence of visual defects (Costa et al., 2011). However, in the last decades, it has emerged that, in addition to the subjective appearance evaluation of agricultural products, volatile organic compounds (VOCs) play a key role in agro-industrial processes and all food-related sciences and technologies. Indeed VOCs are at the origin of food aroma and flavor, and hence, their content and composition directly influence food perception and acceptability by consumers (El Hadi et al., 2013).

Each food product has a characteristic aroma depending on the combination between concentration and threshold of perception of the individual VOCs. However, due to the complex nature of VOCs profiles, their evolution in time and the interaction with people continuously changes during the food production chain, that is, at the crop stage, during fruit ripening and maturation, and in food processing and storage (Biasioli et al., 2011). Therefore, it is now well established that VOCs can be successfully used as a noninvasive tool for food quality characterization (including consumer acceptability) and for process monitoring in the agro-industrial production chains (Aparicio and Harwood, 2013).

The human perception of volatile compounds is of great importance in evaluating food quality. The olfactory system binds together odor molecules and can detect odors at a parts per trillion level using between 10 and 100 million receptors (Deisingh et al., 2004). Its disadvantage is that no two brains are alike, and the same brain may react differently in situations depending on the individual's health, mood, or environment, making the data subjective (Baldwin et al., 2011). In this sense, substantial efforts have been made to improve aroma detection for quality control of agricultural products through analytical methods (Lozano et al., 2006). Gas chromatography is currently considered the reference method for VOCs analyses in food, but despite its high sensitivity [with suitable pretreatment and preconcentration stages, gas chromatography mass spectrometry (GC–MS) systems can reach detection limits as low as 0.1 pptv], this technique is expensive and time consuming (Biasioli et al., 2011). Therefore, alternative technologies have been developed, mainly operating on a principle similar to the human nose, such as the electronic nose (e-nose) and the proton transfer reaction–mass spectrometry based on a time-of-flight mass spectrometer (PTR–TOF–MS; Ionicon, Innsbruck, Austria). These instruments clearly do not replace but provide complete conventional analyses of volatile compounds by sensory methods and by traditional analytical techniques (Schaller et al., 1998; Taiti et al., 2015a).

The e-nose is an instrument, which comprises an array of electronic chemical sensors with partial specificity and an appropriate pattern recognition system, capable of recognizing simple or complex odors (Gardner and Bartlett, 1994). This is a nonspecific instrument able to identify differences in VOCs but not able to identify chemical compounds. Normally it is not sufficiently accurate to monitor changes in VOC profiles. On the other hand, the PTR–TOF–MS,

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compared with the e-nose, allows rapid detection and real-time analysis of VOCs, enabling compounds' identifications and monitoring during the food chain (Biasioli et al., 2011; Sulzer et al., 2012). Its first version was initially used to study VOCs into the air, being now grown exponentially and used for a wide range of applications (Lindinger and Jordan, 1998).

Traditionally both products' and production processes' quality control are performed via physicochemical measurements (mainly spectroscopic), notwithstanding the extreme importance of aroma as a conformity indicator (Ampuero and Bosset, 2003). Therefore, e-noses and PTR–TOF–MS have been used to assess the smell of various agricultural products, such as olive oil (Taurino et al., 2002), fruit (Taiti et al., 2015b), vinegar (Anklam et al., 1998), vegetables (Taiti et al., 2015a), and apples (Soukoulis et al., 2013). Indeed, both e-nose and PTR–TOF–MS measure the VOCs by directly sampling the air to be examined, without any preliminary sampling preparation. Both instruments require a multivariate data analysis for the interpretation of the complex data sets. This aspect will be examined in the "multivariate approaches" section.

Foodstuff freshness, particularly the determination of shelf life, is another application suitable for these instruments (Labreche et al., 2005). Also, for postharvest quality control and maintenance, such technologies offer the great opportunity for detecting specific volatile biomarkers at early stage of deterioration (Pallottino et al., 2012; Soukoulis et al., 2013). In fact, the use of VOC production patterns, specific for different food spoilage microorganisms, would permit a company to take full advantage in order to prevent fruit losses during storage and transport, by making adequate decisions (enabling remedial measures for dynamic control), for example, in the case of changing a harbor destination to the nearest one, for a rapid commercialization of goods.

The multiple uses of these tools in the agricultural field, and particularly postharvest quality control and cultivar classification, will be displayed focusing mainly on applications regarding citrus fruits and dairy products. Two technical sections on PTR–TOF–MS and multivariate approaches will be reported.

## 8.2 ELECTRONIC NOSE APPLICATION TO CITRUS FRUITS

Nowadays the attention to e-nose technology application to citrus fruit has greatly grown. This is due to the need of a rapid and early detection of the metabolic alterations owing to fungal pathologies and a reliable and accurate method, able to implement and manage an effective monitoring system as a part of a quality assurance program.

The most common and serious diseases which occur in Italy during storage and marketing, as well as in many other countries, with the consequence of significant economic losses, are green and blue molds, incited respectively by *Penicillium digitatum* Sacc. and *Penicillium italicum* Wehmer. These two destructive fungi attack severely blood oranges and lemons picked in late winter and early spring. Minor decay such as sour rot (*Geotrichum citri-aurantii* Link ex Pers.) and brown rot (*Phytophthora* spp.) can become a problem if heavy rain falls in the last stages of fruit growth (Lanza and Strano, 2009).

When citrus fruit are wounded, they produce a range of VOCs, such as limonene, β-myrcene, α-pinene, sabinene, acetaldehyde, ethanol, ethylene, and $CO_2$. Most of them have pronounced stimulatory effect on germination of Penicilli spores, representing an early indication of the upcoming deterioration (Droby et al., 2008). Moreover fungi produce volatile compounds as they start colonizing a substrate (Kaminski et al., 1974). These volatile fingerprints vary with individual microorganisms being characteristic and different from those produced by bacteria. Mold spoilage, altering flavor, odor, appearance, as well as shelf life of citrus fruit render the product unsafe and unacceptable. When sporulation covers the fruit, it is easy to avoid the consumption, but this is difficult at early deterioration stages. For this reason, more efficient and standardized methods for quality control of citrus fruit are needed.

E-nose technology, based on semiselective gas sensor arrays for the detection of VOCs, have provided many benefits to a variety of industries, including food, packaging, and biomedical ones. Most research efforts related to e-nose employment have been concentrated primarily on aroma analysis and quality control of different products such as meat, grains, coffee, mushrooms, cheese, sugar, fish, and beverages (like beer and orange juice), on various processing conditions. E-nose technology was compared to the conventional technique headspace solid-phase microextraction (SPME)–GC–MS, by linear discriminant analysis (LDA) of volatile compounds measured by both instruments, with the purpose of solving authenticity problems concerning citrus juices' origin (Reinhard et al., 2008). Encouraging results were obtained in terms of reliability, reproducibility, sensitivity, and applicability of both technologies. E-nose was evaluated for its capacity to differentiate unpasteurized and pasteurized orange juice samples processed at three different regimes, obtaining, only in some cases, satisfactory results in distinguishing the different juices (Bazemore and Rouseff, 1998). The efficacy of e-nose was studied to evaluate and classify three types of commercial orange juice samples (Shaw et al., 2000). Using a discriminant analysis, the e-nose was compared to headspace gas chromatography (HSGC) showing a lower classification ability. Shaw and coworkers considered the two analytical techniques complementary, since they obtained different separation patterns, concluding that an additional volatile constituent not quantified by HSGC influenced the electronic sensors.

Other studies on citrus fruit using e-nose technology regarded the metabolic changes as a consequence of

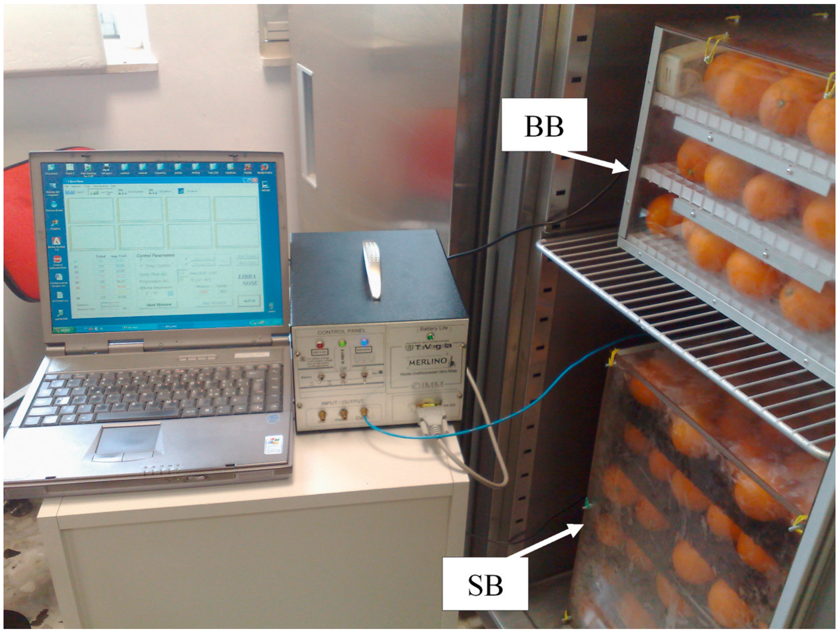

**FIGURE 8.1** **E-nose and PC used for testing, connected to the blank box (BB), where pathogen was absent, and the sample box (SB) containing a defined percentage of inoculated fruit placed among sound fruit.**

respiration, transpiration, and/or fermentation of fruit during storage. Although the postharvest fungal disease detection of citrus under cold storage conditions is important, few publications are present.

The aroma variation of oranges was studied during a storage period of a month by principal component analysis (PCA) and partial least squares discriminant analysis (PLS-DA; Di Natale et al., 2001). Results have evidenced a good sensitivity and resolution of e-nose sensors to measure the aroma of decay of oranges and to correctly predict the storage days. The possibility to detect the presence of the volatile compounds released by *Penicillium* spp. on single fruit, previously inoculated ($1 \times 10^7$ conidia/mL), after 6 days, using a commercial e-nose was studied (Menesatti et al., 2006). The results about a commercial e-nose's capability to discriminate between lemon and oranges noncontaminated and contaminated with *P. digitatum* spores was reported (Pallottino et al., 2009, 2012). Moreover, the early detection of low volatile compounds' production was assessed in infected citrus fruit, placed in controlled environment (Fig. 8.1), in combination with a PLS-DA (Menesatti et al., 2013). Results evidenced high specificity and sensitivity of all the models tested and low levels of infection obtained high percentages of correct classification.

Recently, it was evaluated the efficacy of an e-nose, composed of four sensors made of different organic conductive polymers, for a fast and early detection of fungal activity and fruit biodeterioration on oranges inoculated with *P. digitatum* (Gruber et al., 2013). The results demonstrated that the instrument was able to carry out an analysis time in 40 s and significant responses were found after only 24 h of incubation; a very useful result considering the advantages that the food industry could receive from the application of a rapid response technique in quality control.

## 8.3 ELECTRONIC NOSE APPLICATION TO DAIRY PRODUCTS

The e-nose was defined as "an instrument which comprises an array of electronic chemical sensors with partial specificity and an appropriate pattern recognition (PR) system, capable of recognizing simple or complex odours" (Gardner and Bartlett, 1994). Sensor arrays and PR tend to predict the quality of a sample without providing hard data with respect to composition and concentration (Krantz-Rülcker et al., 2001). Therefore, the main applications to milk and dairy products are shown.

The e-nose is able to discriminate the pure milk from adulterated milk. E-nose was used in order to discriminate among three types of regularly distributed skimmed milk such as pure, added with reconstituted milk, and watered milk, applying both LDA and PCA (Yu et al., 2007). Moreover, the measurement generated by the e-nose can be used to detect both bacteria growth in milk and its shelf life (Labreche et al., 2005). The sensors' response was associated with features of milk flavor, changing over time, related with the bacterial load. Moreover, an e-nose "real-time operating system" was developed for raw milk quality discrimination assessing the concentrations of different VOCs due to microbial contamination and chemical reactions (Sivalingam and Rayappan, 2012).

Concerning the applications on cheese, the e-nose, based on metal oxide semiconductor (MOS) sensors coupled with ANN (artificial neural network), was used to for the classification of Pecorino cheese according to its ripening time and processing (Cevoli et al., 2011). E-nose technology was easily adapted also in monitoring the smell changes occurring during the ripening process of Danish blue cheese (Trihaas and Nielsen, 2005). Another application that may be considered successful is the evaluation of cheese shelf life. E-nose was adopted by Benedetti et al. (2005) to analyze the complex evolution of the aroma profile of Crescenza cheese in order to define the proper shelf-life range. The change of the fingerprint during storage is a useful criterion to define or confirm the hypothesis of shelf-life dating. In another experiment, the evaluation of the odor profile by the e-nose system allowed an objective and rapid measurement of volatiles that could be easily related to the shelf life for routine quality control of fresh cow stretched-curd cheese, as well as sheep and goat cheese obtained from a small dairy in Sicily (Conte et al., 2011).

E-nose was utilized in order to recognize the origin of the cheese on a nutritional or geographical basis. Examination of milk from cows, grazing Alpine clover, and red fescue pastures, demonstrated the successful use of this device for routine control analysis as a tool for the recognition of the animals' diet botanical origin for protected designation of origin (PDO) dairy products (Falchero et al., 2009). Moreover, a study on the volatile compounds of Emmental cheese of different origins demonstrated the suitability of e-nose as tool to identify the geographical origin of this cheese: PCA achieved 90 and 91% of correct classifications for the cheese from Switzerland or other regions, respectively (Pillonel et al., 2003).

Several studies compared the e-nose to panelists for cheese evaluation by the triangular test. E-nose evaluation was comparable with that of the triangular test in a study aimed to identify the flavor characteristics of sheep cheeses made with raw milk or thermized milk added with starters, and milk from animals fed with different amount of extruded linseed (Branciari et al., 2009).

In the last decade, the Research unit for the extensive animal husbandry (CREA-ZOE) used the e-nose for its quick ability of discrimination, not requiring any samples' pretreatment. Most of the results are under publication.

Some results are reported as follows.

1. Milk classification: The e-nose was able to discriminate milk from three species (cow, sheep, and goat). In Fig. 8.2, the samples from different species (sheep and goat) are classified and placed distant, while within the species, breeds are placed nearby.
2. Classification for age of cheese: The e-nose has distinctly differentiated Scamorza cheeses 15 days old compared

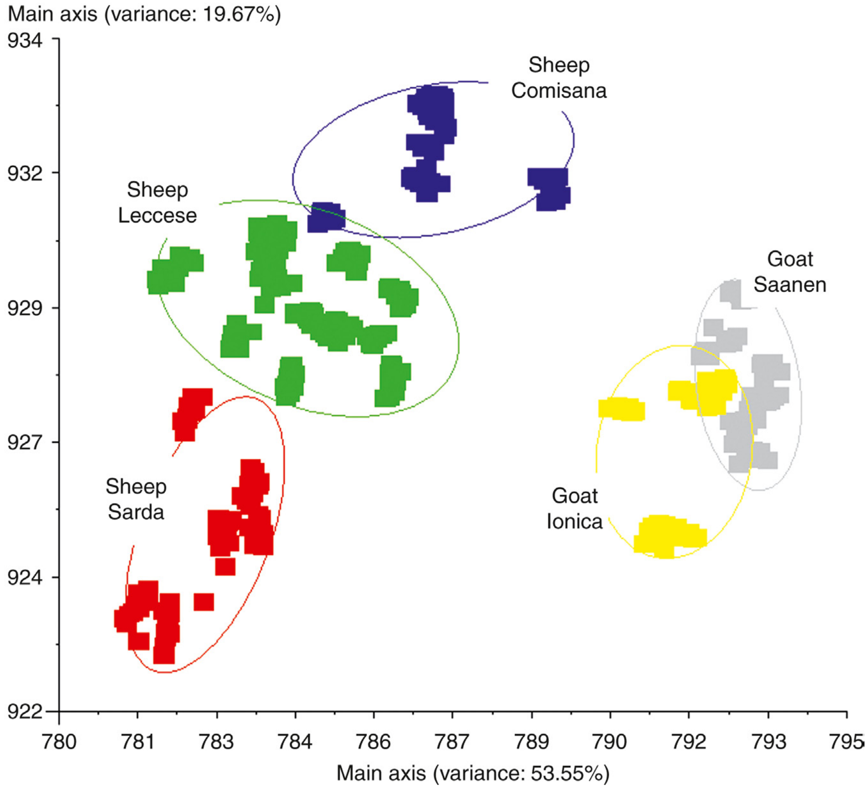

**FIGURE 8.2** LDA, where milk samples are well separated for species and breeds.

to the cheeses produced the same day but after 30 days of ripening.

3. Shelf life of mozzarella cheese: The e-nose clearly subdivided into two classes of 0 and 5 days old samples of mozzarella cheese, but only those produced by artisan dairies; this is likely due to industrial technology effects on shelf-life.
4. Differentiation of fresh and frozen products: The olfactory prints of fresh and frozen mozzarella cheeses were compared. The e-nose was able to distinguish the fresh mozzarella from that frozen and thawed. GC–MS analysis showed a significantly different composition of the volatile compounds (acetoin, limonene, eucalyptol, and *p*-cymene) in the fresh cheese compared to the frozen one. While the e-nose rapidly showed the differences, the GC–MS allowed understanding the causes.
5. Watered milk discrimination: A trial compared milk with watered milk samples at 20–50 and 100%. E-nose could distinguish the different samples, although the discrimination was lower among the samples with lower percentages of water.
6. Differentiation on diet supplementation: The e-nose was able to correctly classify the milk samples on a concentrate supplementation basis (linseed, destoned olive cake, sunflower seed, etc.).

Although e-nose does not identify the volatile compounds, it does perform comparisons, and after adequate statistical analyzes, it indicates whether the sample belongs to the same class of samples. Consequently, it is less complete than the GC–MS, but it is faster and cheaper and does not require any particularly skilled operator or sample preparation.

## 8.4 PTR–TOF–MS TECHNOLOGY AND ITS APPLICATIONS

The PTR–MS is an innovative tool that enables the real-time separation and quantification of VOCs in complex gas mixtures, from trace levels up to parts per million (for more details, see Lindinger and Jordan, 1998; Blake et al., 2009). Very briefly, the common version of the PTR–MS consists of a hollow cathode ion source that produces an intense and pure $H_3O^+$ ion beam driven by a homogeneous, relatively high, electrical field through a drift-tube reactor, where the volatile mixture to be measured is directly admitted, usually without any pretreatment, and where eventually all volatile compounds are ionized by proton transfer from $H_3O^+$, and the protonated VOCs will then be mass analyzed (for more detailed technical information, see Blake et al., 2009). $H_3O^+$ is a suitable reagent ion for detection of VOCs in air because most VOCs undergo an efficient proton-transfer reaction with $H_3O^+$, whereas the major component of clean air does not, given that their reaction with $H_3O^+$ is not thermodynamically favorable. The key advantages of these instruments that have considerably boosted its use in a wide range of fields (including in food science and technology), are: (1) an unprecedented number of VOCs can be measured simultaneously with very high time resolution within a large dynamic range (Jordan et al., 2009) and (2) there is no need for pretreatment of the sample (Jordan et al., 2009; Blake et al., 2009). In addition, the PTR–MS bases its operation on the concept of a soft chemical ionization to protonate volatile compounds with a proton affinity higher than the water one (proton affinity of $H_2O$: 166.5 kcal/mol); indeed $H_3O^+$ does react with most VOCs at relatively low energies, resulting in a lower degree of fragmentation when compared with chemical ionization through more energetic reactions or hard ionization techniques such as electron ionization mass spectrometry (Lindinger and Jordan, 1998; Jordan et al., 2009). However, the PTR–MS is a one-dimensional analytical method and only the nominal mass-to-charge ratio of the protonated parent ion can be determined, therefore, resulting in difficulties or the inability to unambiguously identify the studied VOCs (Jordan et al., 2009). To overcome some of the limitations of the PTR–MS, recently it was coupled to a high-resolution TOF mass analyzer that, unlike the PTR–MS that couples to the reaction tube of the PTR a quadrupole for the separation of the masses, uses an electric field to accelerate the ions and a detector to measure their speed (Blake et al., 2009). This has enabled a higher sensitivity (down to single digit parts per trillion volume), time resolution (0.1 s), and a higher mass resolution that allows the discrimination between isobaric peaks at a center of mass separation (Jordan et al., 2009; Blake et al., 2009). The analytical potential of PTR–MS has further increased; thanks to the recent coupling of PTR–MS instruments with switchable reagent ion system that can produce, through the hollow cathode source, different parent ions (eg, $H_3O^+$, $NO^+$, and $O_2^+$; Jordan et al., 2009). The different ionization induced by different precursor ions not only allows, in some cases, the separation of isomeric compounds (Sulzer et al., 2012), but also enables the ionization of molecules with a proton affinity lower than water, and therefore not seen by proton transfer ionization (Blake et al., 2009).

Thanks to these technical features, the PTR–TOF–MS has a high analytical throughput, which provides mass spectra with a high informational content and a high time resolution (Blake et al., 2009). These features make this instrument particularly suited to online analysis of dynamic flavor release (both in vitro and in vivo) from diverse food matrices along the food-to-fork production chain (ie, from plants and crops to food processing and storage and, eventually, during food consumption; Biasioli et al., 2011). Following its technological developments in the last decade, the PTR–MS is now considered an established method for the rapid, nondestructive VOCs' detection in a wide range of fields, including the entire food-to-fork chain, as clearly shown by the number of studies conducted in the food research area (Fig. 8.3; Biasioli et al., 2011; Sánchez Del Pulgar et al., 2013). As a result, the PTR–MS–TOF has been extensively used for classification studies of a broad range of food products, ranging from bread, truffle, coffee, olive oil, dry-cured hams, and intact fruits to their derivatives (Sánchez Del Pulgar et al., 2013; Gloess et al., 2014; Taiti et al., 2015a,b). As a clear example of the potentialities of this instrument in VOCs detections and analyses along most stages of the food-to-fork production chain, the PTR–TOF–MS was recently used to discriminate between coffees from different origins that were roasted to different roast degrees and along varying time temperature roasting profiles (Gloess et al., 2014). In another instance, the PTR–MS–TOF analyses enabled the discrimination of Iberian dry-cured hams based on the rearing systems, based on the volatile profile emitted by hams from pigs fattened outdoors on acorn and pasture or on high-oleic concentrated feed (Sánchez Del Pulgar et al., 2013). Remarkably the potentialities of this instrument are likely to expand further from the analyses along food-to-fork chain. Indeed with this instrument it is not only possible to monitor in real-time VOC releases, key in aroma perception, from food during eating or drinking (Romano et al., 2014), but it is also possible to evaluate the long-term effects of different diets by PTR–MS breath analysis (Aprea et al., 2012). For example, the PTR–MS–TOF was used to pinpoint and identify markers related to diet and specific pathologic conditions in rats with dietary-induced nonalcoholic steatohepatitis and modifications induced by coffee addition to the diet (Aprea et al., 2012).

## 8.5 MULTIVARIATE APPROACHES

Data from e-nose and PTR–TOF technologies are multivariate. Those technologies are expensive (sometimes very expensive); so for this reason the data set produced is precious and should be processed with adequate and informative techniques. Unfortunately this is not always the case.

A number of statistical analyses have been used for the application, from the simple, such as, for example, the graphical representation of the individual sensor outputs with time (polar plots or spider plots), to multivariate ordination representation (such as PCA) and hierarchical clustering, to the most sophisticated approaches, such as class-modeling and neural networks (Hodgins and Sirnmonds, 1995).

Multivariate ordination techniques are unsupervised techniques which are (and should mainly be) used as exploratory data analyses. Ordination techniques put in order the objects, described by multivariate variables, so that similar objects are near each other and dissimilar objects are farther from each other. These relationships between the objects, on each of several axes (one for each variable), are then characterized numerically and/or by graphically outputs (Johnson and Wichern, 1992). The mostly used approach, based on eigenvalues and eigenvectors, is the PCA.

Also cluster analysis or clustering is an unsupervised technique; this task is grouping a set of objects, described by multivariate variables in such a way that objects in the same group (cluster) are more similar to each other than to those in other clusters. The most common way to represent hierarchical clustering is by a dendrogram. A dendrogram is computed from a matrix of distances (or similarities) using different kinds of algorithms (simple linkage, complete linkage, etc.)

Discriminant and classification analyses answer to the general question: "Which is the most probable category in which object O could belong (or could be classified)?" This general question expects the presence of at least two categories (groups or clusters), and the object could belong to one and just one group. Discriminant analyses are supervised techniques used to distinguish distinct sets of observations and allocate new observations to previously defined groups. The most commonly used discriminant analyses are LDA, the quadratic discriminant analysis (QDA), and the discriminant function analysis (DFA). Other most sophisticated techniques, such as the soft independent modeling of class analogy (SIMCA) and the partial least squares (PLS), could be (and often are) used only in the classification fashion.

The problem stems for the number of data points needed to adequately represent a data set with a high number of features; it is quite possible that within high dimensional data, clusters exist in separate subspaces. All classifiers can suffer from the curse (Scott et al., 2006). For these reasons, class-modeling approaches have been recently developed and applied. Multivariate class-modeling techniques answer to the general question of whether an object O, stated of class A, really belong to class A (Forina et al., 2008; Abramo et al., 2015). This is a typical question that is addressed in the traceability of PDO foods or in multivariate quality control. On the contrary, the classification techniques assign objects to one of the classes in the problem. For example, LDA assigns an object to the class with the maximum posterior probability (Biasioli et al., 2003). However, these classification techniques are not very useful in the control of quality, variety, origin, or genuineness of a sample when considering their VOC profiles (Granitto et al., 2007; Cappellin et al., 2011). Nevertheless, almost all research papers on food control use classification techniques; furthermore, also when a class-modeling technique is applied, the attention is focused on its classification performance rather than on its modeling characteristics. Class-modeling techniques calculate the "prediction probability" with a classification threshold for each modeled class. Using a class-modeling approach, it is possible to attribute objects not only into one or more classes, but also to none (ie, in this case, the object is an outlier) (Abramo et al., 2015).

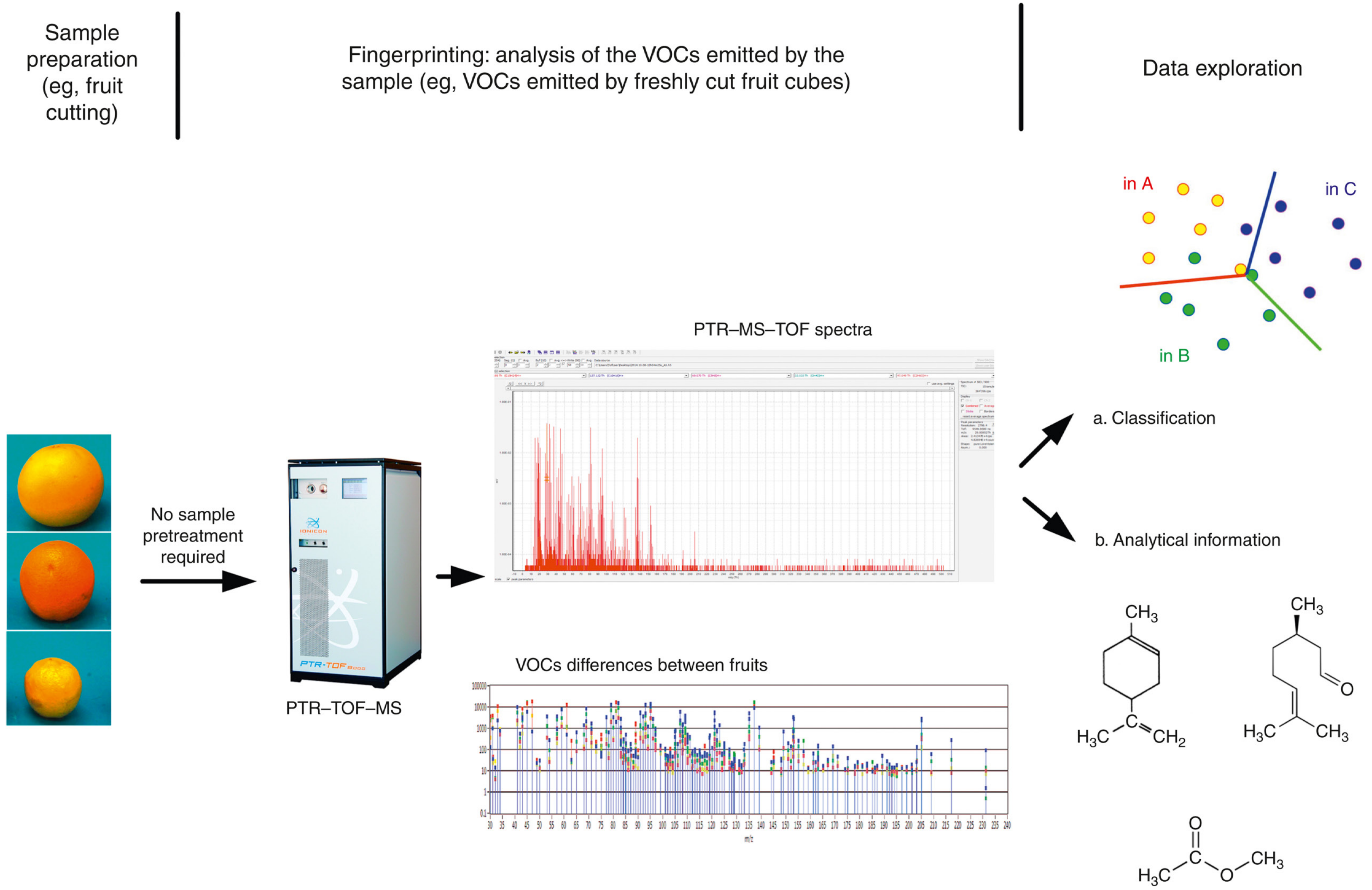
Sample preparation (eg, fruit cutting)
Fingerprinting: analysis of the VOCs emitted by the sample (eg, VOCs emitted by freshly cut fruit cubes)
Data exploration
No sample pretreatment required
PTR–TOF–MS
PTR–MS–TOF spectra
VOCs differences between fruits
100000
10000
1000
100
10
1
0.1
30 35 40 45 50 55 60 65 70 75 80 85 90 95 100 105 110 115 120 125 130 135 140 145 150 155 160 165 170 175 180 185 190 195 200 205 210 215 220 225 230 235 240
m/z
in A
in B
in C
a. Classification
b. Analytical information
CH3
H3C
C
CH2
CH3
O
H3C
CH3
O
C
H3C
O
CH3

**FIGURE 8.3** **Schematic representation of sample analyses and classification using the PTR–TOF–MS.** This technique allows rapid and nondestructive VOCs detection throughout the entire food-to-fork chain (eg, fruits) without any sample pretreatment. The large data set produced by the PTR–TOF–MS is then later used to extrapolate analytical information regarding the product and for classification using different multivariate analyses.

ANNs are used to estimate or approximate functions that can depend on a large number of inputs and are generally unknown. ANNs are generally presented as systems of interconnected "neurons," which can compute values from inputs and are capable of learning thanks to their adaptive nature. ANNs are particularly useful in performing for nonlinear responses (Baldwin et al., 2011).

All discriminant, class-modeling, and ANNs are supervised techniques. Supervised approaches are affected by overfitting. For this reason, two different kinds of strategies should be applied to the statistical analytical procedure. The first strategy is cross-validation, that is commonly used. This approach cross-validates splitting the data into groups, named CV (cross-validated) groups. This procedure involves the removal of one CV group in a random way from the data set, and the model is built with the remaining samples, then the removed CV group is included in the model and the class membership of the samples belonging to the CV group is predicted. This process is applied until all the CV groups are removed once. The performances are calculated during the cross-validation technique and at least the model was built using the whole data set. The second strategy to avoid overfitting is partitioning. Unfortunately this strategy is not commonly used. This strategy separates the data set into at least two groups, one to build and cross-validate the model and the second to internally to test the model. The methodologies used for partitioning are based on random sampling or using specific algorithms (Kennard and Stone, 1969; Galvão et al., 2005). Random sampling is the most used approach, but this kind of approach should be applied many times on the same data set so the results could be complex.

## 8.6 FUTURE TRENDS

Currently, most of the traditional measuring techniques used to determine food quality are destructive (eg, texture, firmness, total soluble solids, acidity, color score, juice content) and involve random sampling, which increases the likelihood of an incorrect evaluation. Thus, nondestructive techniques, based on aroma characteristics, can offer the possibility to optimize food quality assessments.

E-nose technology is attractive for several reasons; however, there are still few industrial applications (Loutfi et al., 2015). This is mostly due to the difficulties in reproducibility of the sensors and the need of pattern recognition algorithms, which can manage the signal analyses. The progress in e-nose technology requires and coincides with an increased understanding of the biological mechanisms of human olfactory system and, with further research, a wider application of e-nose technology in the food industry is predictable for the future. For instance, the e-nose technology shows promise for future applications in early, rapid, and nondestructive tests to monitor continuously the qualitative conditions of perishable goods stored. Indeed, hypothetically, the e-nose could be successfully applied in cold storage rooms, as a nondestructive and noncontact indirect technology for the early diagnosis of fruit molds and to prevent the spread of fungal growth during storage and transport.

In the last decade, the PTR–MS tools have also emerged as new and promising tools that can guarantee food analyses with high informational content and a high time resolution. These two key features make this technology particularly suited to the online analysis of dynamic flavor releases from diverse food matrices along the food-to-fork production chain. Indeed, the PTR–MS represents a noninvasive tool capable of providing fingerprint for agro-industrial product characterization (eg, based on genetics or origin) and on the other hand, provides quantitative and qualitative information on the VOCs involved during food production, processing, and consumption. In conclusion, PTR–MS will play a pivotal role in future understanding of the food production and consumption processes, opening new promising fields of application in food research, thus providing key elements to improve food production processes and enhance food consumer acceptability.

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## Chapter 9

# Olive Oil and Electronic Nose

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## 9.1 INTRODUCTION

Virgin olive oil is the olive oil obtained directly from olives and solely by mechanical means under conditions that do not lead to alter the oil composition. Solvent extraction, re-esterification processes, and the blending with other vegetable oils is prohibited (EEC, 1991).

Chemical and sensorial quality standard allow to discriminate olive oils into three groups, namely, extra virgin (EVOO), virgin (VOO), and *lampante* (LOO). The quality of the oil is strictly correlated with that of the fruits, primarily, by the harvesting systems, the techniques of extraction, the kneading of the olive paste, and the separation of the oily phase. EVOO contains a great number of volatile and nonvolatile compounds, including phenolic compounds responsible for its fragrant and peculiar flavors. These substances also contribute to the stability of the oil.

Volatile compounds present in VOO have been extensively studied (Olias et al., 1993; Flath et al., 1973). More than 280 compounds have been identified in the volatile fraction of VOO. They include hydrocarbons, alcohols, aldehydes, ketones, acids, esters, ethers, furan derivatives, thiophene derivatives, pyranones, thiols, and pyrazines (Angerosa et al., 1999; Guth and Grosch, 1993; Solinas et al., 1987). However, only a fraction of them is present at concentrations higher than their odor threshold limit and, thus, contributes to the resulting aroma of olive oil. The qualitative composition of EVOO obtained from healthy fruit, harvested at the proper ripening stage, and with correct extraction techniques results in a typical aroma profile (Kiritsakis and Min 1989; Salch et al., 1995). This profile is primarily derived from the decomposition of linoleic and linolenic acid through the lipoxygenase pathway, which generates C6 volatiles aldehydes and alcohols, such as hexanal, (E)-2-hexenal, (Z)-3-hexenal, hexan-1-ol, (Z)-3-hexen-1-ol, hexyl acetate, and (Z)-3-hexenyl acetate together with a few of C5 aldehydes, ketones, and alcohols (Olias et al., 1993). Therefore, the quantitative analysis of the aroma profile of EVOO is a way to monitor changes in the linoleic and linolenic acid composition or modification of the lipoxygenase activity, which in turn may reveal oil of poor quality.

Among the techniques used to determine the aroma profile of olive oils, the most important is the sensory evaluation because it is directly connected to the consumers' organoleptic judgment of oil quality (EEC, 2002). However, sensory evaluations generally lack precision and reproducibility. In addition, methods based on trained panels are time consuming, tedious, and expensive and they are not always available. For this purpose, advancements of analytical instruments have stimulated researchers to develop new methods for the evaluation of sensory qualities of fats and oils (Guadarrama et al., 2000).

Gas chromatography provides a rapid accurate means of determining the fatty acid distribution of VOO. This technique is essential for product development, process control, and marketing because it is well known that the physical, chemical, and nutritional characteristics of oils are influenced by the fatty acid composition and their esterification with glycerol. The cost of the instrumentation, the need of high-purity and hazardous gas carriers, the extensive preliminary samples treatments and derivatization, the need of frequent calibrations, and the need of trained personnel limit the use of such technique to qualified laboratories only.

An alternative analytical approach that tried to overcome the aforementioned drawbacks was introduced at the beginning of the 1990s with the concept of "electronic nose." Electronic noses were novel analytical devices intended to the rapid and untrained analysis of aroma profile. Gardner and Bartlett (1993) defined the electronic nose as "an instrument, which comprises an array of electronic chemical sensors with partial specificity and appropriate pattern-recognition system, capable of recognizing simple or complex odors." Nowadays, several commercial intelligent gas sensor array instruments are now available on the market covering a variety of chemical sensor principles, system design, and data analysis techniques. Operationally, an electronic nose is a "sensing system" comprised of three parts: a sampling system, a

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detector producing an array of signals correlated with the chemical composition of a gas, vapor, or odor, and an appropriate pattern classification system.

In this chapter, we present the analyses conducted on the EVOO with two different commercial electronic noses, respectively, an electronic nose based on metal oxide semiconductors (MOS) and metal oxide semiconductor field effect transistors (MOSFET) gas sensors and an electronic nose based on proton transfer reaction-mass spectrometry (PTR-MS). In the following sections, the basic principle of these two systems will be presented, followed by a review of the pattern recognition techniques used to translate their analytical signal into quality attributes. Finally, four case studies employing these techniques on relevant research questions in olive oil technology will be discussed.

### 9.1.1 Electronic Noses Based on MOS/MOSFET Gas Sensors

Former electronic noses were assembled with an array of various kinds of gas sensors. Nowadays there are four main technologies to produce gas sensors: MOS, MOSFET, conducting organic polymers (CP), and piezoelectric crystals (bulk acoustic wave, BAW; surface acoustic wave, SAW). Other sensors, such as fiber optic, electrochemical and bimetal sensors, are still in developmental stage and may be integrated in the next generation of the electronic noses. In all cases, electronic noses based on gas sensors aim to create an array of a specific sensing elements to provide a characteristic fingerprint of the product's aroma (Mannino et al., 2007). Such gas sensor arrays should be sensitive toward chemical compounds similarly to the human nose, respond to different compounds present in the headspace of the sample, and be highly stable, reproducible, and reliable. Unlike gas chromatographs, electronic noses should show short reaction and recovery time and guarantee easy calibration and simple data interpretation. Furthermore, electronic noses should be portable and have reduced footprint and power requirements (Schaller et al., 1998).

The first electronic nose presented here consists of a commercial apparatus (model 3320 Applied Sensor Lab Emission Analyzer, Applied Sensor Co., Linkoping, Sweden), comprising an automatic sampler carousel, a detector unit equipped with MOS and MOSFET sensors, and software for data recording and pattern recognition. Twenty-two sensors compose the sensor array: 10 sensors are MOSFET and 12 are MOS. The MOSFET sensors are divided into two arrays of five sensors each, one array operating at 140°C and the other at 170°C, while the MOS are kept at 400–500°C during all the process phases. The automatic sampling system supports a carousel of 12 sites for loading the samples and permits the control of internal temperature. The analysis is performed on aliquots of 1 g of sample, which is introduced in 40 mL Pirex® vials with a pierceable silicon/Teflon disk on the cap. The assay starts with the incubation of the sample at 40°C for 10 min for headspace equilibration. Then, an automatic syringe samples the headspace and a pump transfers the volatile compounds over the sensor surfaces. Signal recording lasts after 60 s. Each sensor is then exposed to filtered air at a constant flow rate (60 mL/min) to bring the gas sensor signal back to the baseline.

### 9.1.2 Electronic Noses Based on Proton Transfer Reaction-Mass Spectrometry

The simplest type of mass spectrometry based e-nose consists of a gas chromatograph-mass spectrometer device where a short capillary column is employed as a transfer line to the mass spectrometer. Similarly to classic electronic noses, this type of instrument provides a fingerprint of the analyzed sample, which can be used for classification or process monitoring purposes (Pérez Pavón et al., 2006). Over the years, other MS-based techniques have been developed with the aim to increase the informational content of the analytical output. A particular successful example of this technical advancement is represented by PTR-MS, which allows rapid, direct, and highly sensitive online monitoring of volatile organic compounds (VOCs) in food (Biasioli et al., 2003; Cappellin et al., 2013; Aprea et al., 2006). PTR-MS has been described in detail by Lindinger and coworkers (Lindinger et al., 1998a, 1998b). A typical PTR-MS output consists of a mass spectrum, composed by several mass peaks, having different mass-to-charge ratios and intensities. First introduced by Lindinger and coworkers (Hansel et al., 1995) as a multipurpose gas analyzer, PTR-MS now counts a wealth of applications in the food sector (Biasioli et al., 2011a). A detailed description of the PTR-MS working principle was reviewed recently (Biasioli et al., 2011b). Briefly, in PTR-MS, the gaseous sample is introduced at a constant flow through a heated transfer line (Fig. 9.1). The gaseous mixture then reacts with a pure beam of hydronium ions ($H_3O^+$); most compounds will then undergo the following reaction:

$$H_3O^+ + M \rightarrow H_2O + MH^+$$

As in other MS-based techniques, ionization is essential to the following separation and detection steps, performed by the mass analyzer. Many types of ionization techniques have been developed, involving chemical reactions, plasma, laser, and so forth; one of the benefits of proton transfer using hydronium ion is that the reaction is thermodynamically favored with most VOCs, while most inorganic volatile species (including oxygen and nitrogen) do not undergo protonation, thus allowing the direct analysis of VOCs present in trace amounts in air without the use of a dilution buffer. Another key advantage of proton transfer lies in its character of *soft* ionization. This implies that most analytes

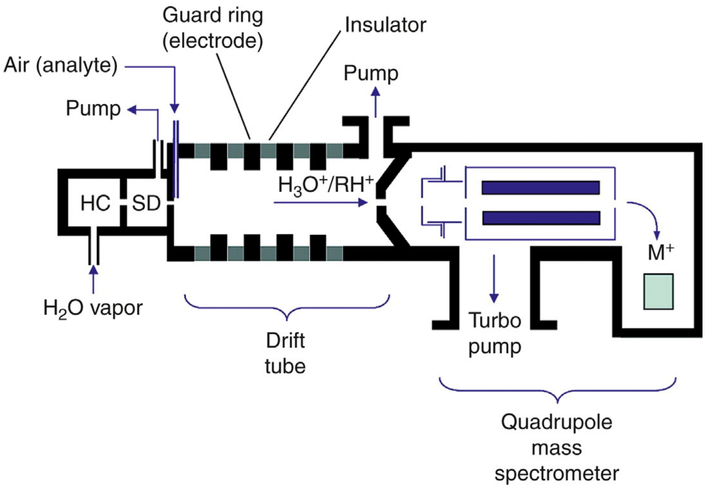

**FIGURE 9.1 Schematic representation of a PTR-MS.** *(Reprinted with permission from Blake et al., 2004. Copyright 2004 American Chemical Society.)*

are protonated without undergoing further fragmentation, providing key information as to the compound of origin.

### 9.1.3 Pattern Recognition and Human Stimuli Analogy

It must be pointed out that any electronic nose methodology, regardless of the signal transduction principle, provides a useful fingerprint connected with the quality, authenticity, or processing only if supported by an appropriate multivariate statistical technique. Pattern recognition routines like principal component analysis (PCA), cluster analysis (CA), partial least squares (PLS), linear discriminant analysis (LDA), and artificial neural network (ANN) are needed to translate the chemical information provided by each of the gas sensors of the electronic nose into new macrovariables, which can be used to express the EVOO quality grade, the geographical origin, the health status of the olives, the presence of extraneous products, or the shelf-life status. The translation of chemical signals into quality attributes is the same activity performed by the brain following to the chemical perception of stimuli cells. As aroma compounds come directly through the nasal passages, they excite a number of stimulus-responsive receptors hosted in millions of sensing cells comprised within the olfactory epithelium. Each receptor uniquely interacts with odorous molecules. Stimulation of these receptors is translated into the language of the nervous system. Hence, through the human experiences, the brain becomes trained to associate specific patterns of neural messages into quality attributes. This experience is then used to predict or judge the quality of novel samples. Similarly, electronic noses, regardless of the sensor type or mechanism of signal transduction, emulate the pattern recognition activity exerted by the brain. The electronic nose reacts to the sample and produces electrical signals that are correlated with its aroma profile. A computer reads the pattern of signals, and interprets them with some form of "intelligent" pattern classification algorithm.

## 9.2 CASE STUDY 1: EVALUATION OF OXIDATION STATUS IN VIRGIN OLIVE OILS

EVOO presents a complex flavor, which can be greatly affected by the storage conditions. Oxidation processes affected by air, heat, light, and metals are mainly responsible for the change in the aromatic profile of olive oils (Angerosa et al., 1999; Morales et al., 1997). Consequently, it is a matter of great concern for the olive oil industry to preserve the positive attributes of EVOO from production to bottling, up to purchasing.

Nowadays, there are different methods used and/or proposed for evaluating the oxidative deterioration of olive oil. Among the routine methods, there are the peroxide value (PV), which determines the amount of primary oxidation products ($meqO_2/kg$) and ultraviolet light absorption at 232 and 270 nm ($K_{232}$, $K_{270}$, and $\Delta K$), that measures the formation of conjugated dienes and trienes due to the formation of secondary oxidation products. According to the European Commission (EEC, 1991; EEC, 2003) and the International Olive Oil Council (IOOC, 2001) regulations, the EVOO oxidation level is assessed by the PV and spectrophotometric absorbance, defining the following limits: PV $\leq 20$ meq/kg and $K_{270} \leq 0.22$, and $\Delta K \leq 0.01$. The EEC legislation also considers that the value of $K_{232}$ must be $\leq 2.4$. Nevertheless, these methods supply only limited information as to the level of olive oil oxidation.

In recent years, high-performance liquid chromatography (HPLC) and gas chromatography coupled with mass spectrometry (GC–MS) were applied to detect changes in the chemical composition of olive oil during storage. HPLC with different detection systems has been used for hydroperoxide analysis (Oshima et al., 1996). GC–MS was used to detect hydroxylated fatty acids and volatile compounds originated from hydroperoxide degradation (Morales et al., 1997) and to identify the products of triglyceride oxidation (Rovellini et al., 1998).

However, these techniques are complex to perform or to interpret, expensive and time consuming, and generally highlight only one or a few aspects of the oxidation process, giving only partial information about the extent of the process. On the other hand, the olive oil industry needs to be able to know quickly the level of oil oxidation in order to predict its remaining shelf life. Moreover, consumers expect manufacturers and retailers to provide products of high quality and look for quality seals and brands. Therefore, the development of innovative analytical tools able to execute fast and reliable quality checks on EVOO is required.

This study reports the potential of electronic nose based on gas sensor array, in combination with multivariate statistical analysis for evaluating the oxidation level, that is, oil quality at bottling time, storage in real-life conditions and without applying an accelerated thermooxidation process. For this study, 61 VOO from typical cultivars of Garda region were packaged in glass bottles and stored in the light for 1 year and in the dark for 1 or 2 years, 1 year being generally considered the maximum storage period from bottling to consumption. This approach could represent a faster recognition tool for monitoring olive oil oxidation since it is characterized by simplicity of sample preparation.

The oils were analyzed before and after storage, using both chemical methods and electronic nose technique. In the literature, there are several examples that demonstrate the possibility of using an electronic nose for the characterization of vegetable oils (Gan et al., 2005; Martin et al., 1999), while information about the use of an electronic nose to predict shelf life of vegetable oils or to monitoring oil oxidation under real-life storage conditions are very limited (Cosio et al., 2007; Shen et al., 2001).

The 61 EVOOs were analyzed before storage. They presented an acidity ranging from 0.1 to 0.3, and PVs from 3 to 6; spectrophotometric indices $K_{232}$ and $K_{270}$ ranged from 0.8 to 1.4 and from 0.08 to 0.15, respectively, whereas $\Delta K$ was always lower than 0.01. The same samples were then analyzed after 1 year of storage under dark (class 1), under light (class 2), and after 2 years under dark (class 3). All samples of the three classes presented an acidity value lower than 0.4, PV from 16 to 22 (class 1), from 17 to 61 (class 2), and from 17 to 39 (class 3). Most samples had $K_{232}$, $K_{270}$, and $\Delta K$ above the lower limits. At the end of their storage period, all VOO were also analyzed by electronic nose. The response of the electronic nose is characteristic of each sensor and depends on the concentration and the profile of the volatile compounds present in the olive oil. The signal obtained with the electronic nose (22 sensors), together with the classical chemical determinations (5 parameters) calculated at the end of the sample storage period were considered all together and analyzed by means of PCA. The first principal component and the second principal component were enough to display the data structure, since they explained 61% of the total variance. Examining the score plot (Fig. 9.2) in the area defined by the first two principal components, a separation of the samples into three groups was found according to the different storage conditions and storage periods. Only a few samples belonging

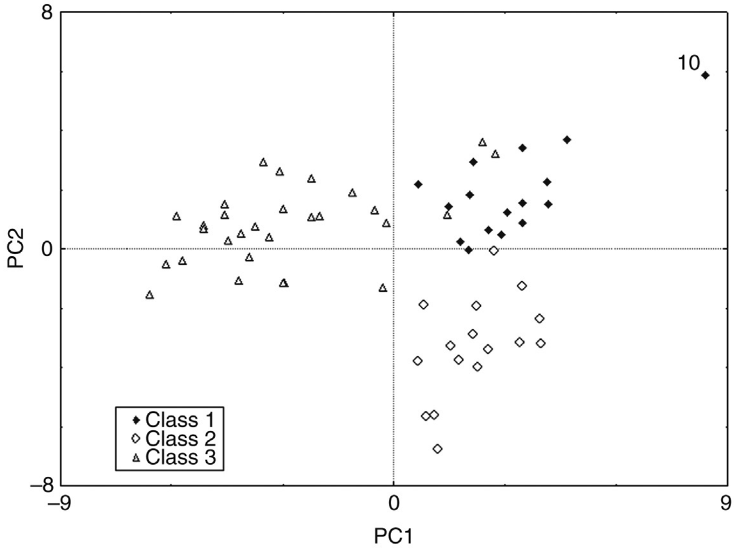

**FIGURE 9.2 PCA on autoscaled data: score plot.** Classes are shown with different symbols.

to class 3 were projected in the middle of class 1, but this does not affect the effectiveness of the plot. Furthermore, based on the position of each group in the plot, it was possible to assign a particular meaning to each component.

The first component was able to separate the oil samples belonging to class 3 (characterized by negative values) from all other samples, that is, the first component was able to characterize the samples on the basis of the storage period. In fact, samples belonging to class 3 were characterized by a storage period of two years, while all the other samples by a storage period of only one year. On the second principal component, oil samples of class 2 had negative values, while all other samples had positive values, that is, the second principal component was able to describe the samples on the basis of the storage conditions. In fact, class 2 samples were stored under light, while all other samples under dark.

Finally, a sample belonging to class 1 appeared far from its class space in the score plot. This sample, labeled in the score plot as sample no. 10, was characterized by the highest scores on the first and the second component. As described before, the meaning of each component is related to the quality of the storage period and conditions. The highest positive scores on the two components were associated to the best storage situation, that is, conservation under dark for one year. The behavior of sample no. 10 confirmed this hypothesis. In fact, all values of the classical chemical parameters for this sample respected the law limits and allowed it to be considered as EVOO. All other samples of class 1 could not be considered as EVOO since they had just UV values or PV values higher than the law limit.

Since the data structure analysis gave a good sample characterization, a classification model was built. LDA analysis was applied to the complete data set to separate the three described classes. LDA applied set gave a recognition percentage of 100%, while only one oil sample was not correctly classified in the validation procedure. Even if this model performed a good classification result, the classification after selection of a minimum number of variables was also considered. For this reason and in order to simplify the classification model by reducing the number of the considered variables, LDA was repeated by considering only the electronic nose data. The classification model gave again 100% correct classification for three classes and also during the leave-one-out cross-validation, all samples were correctly classified (cross-validation error rate of 0%). The discriminant scores for the classification model with the electronic nose features (Fig. 9.3) showed a clear class separation. As expected, the classification model gave the same results as before, that is, a recognition percentage of 100%.

Since an equal classification performance was obtained by considering only the electronic nose sensors, it is evident that chemical analyses were not required in order to achieve a better sample discrimination, that is, chemical analyses did not improve the classification model.

In conclusion, chemical parameters where not relevant when the LDA classification method was applied. In fact, it has been shown how by removing chemical analysis, the classification performance is preserved and a more applicable model is obtained. The final classification model built by means of the electronic nose sensors was able to describe the samples' storage conditions and could represent a simpler, faster, and cheaper recognition tool, since a minor number of variables must be determined.

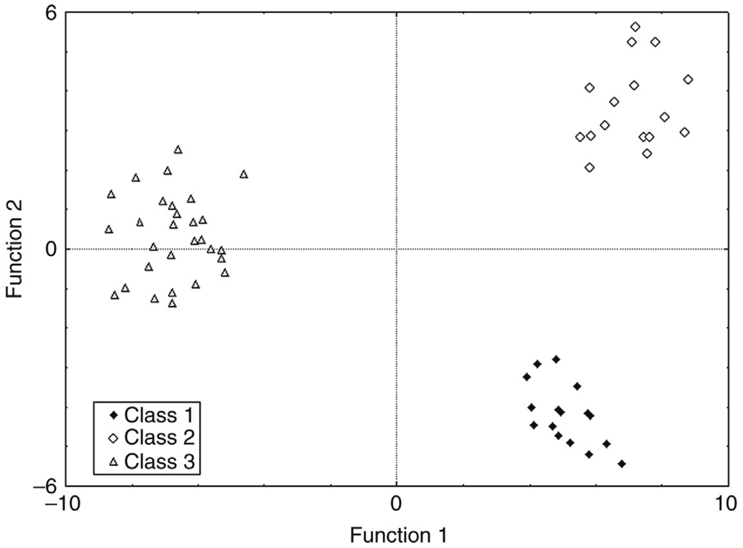

**FIGURE 9.3 LDA classification model with the electronic nose sensors: discriminant scores.** Classes are shown with different symbols.

## 9.3 CASE STUDY 2: CLASSIFICATION OF EVOOs

The quality and uniqueness of specific EVOOs is the result of different factors such as cultivar, environment, and cultural practices. Moreover, an important act of legislation (EEC, 1992) allows the European protected denomination of origin (PDO) labeling of some European EVOOs with the names of the areas where they are produced. This designation guarantees that the quality of the product is closely linked to its geographical origin. PDO olive oils are considered the best among EVOOs based on their authenticity and specified organoleptic characteristics. Therefore, PDO olive oils have a much higher market price and are therefore subject to frauds: the addition of refined oils and/or the marketing of oils from one region as those from another. Consumers are also more and more oriented toward purchasing food products of a certified authenticity and geographical origin.

In the present study, EVOO Garda “Bresciano” has been considered: this is a product made in the Garda Lake, a circumscribed area in the north Italian region of Lombardia and distinguished as PDO since 1997. Detailed percentages of specified cultivar olives, cultural practices, circumscribed geographical production areas, and chemical and sensorial properties are required in order to obtain the PDO label, as indicated in the Production Disciplinary. However, at present no analytical parameters exist that enable the Garda PDO oil to be distinguished from similar products of other regions. The development of precise methods for the classification of oils is becoming very important for the assignment of a “denomination of origin” trademark. Since official analysis of VOOs involves a series of several determinations of chemical and physical constant that will be of little use in the geographical certification of the oil samples, reliable methods of authentication of oil geographical origin are essential.

A variety of analytical methodologies have been proposed for the authentication of vegetable oils, including gas chromatographic analysis (Webster et al., 1999; Cert et al., 2000), nuclear magnetic resonance (Rezzi et al., 2005; Sacco et al., 2000), and mass spectrometry (Caruso et al., 2000). These techniques usually require time-consuming measurements, sample preparation, and a qualified staff. The necessity of quick and simple methods has addressed the present study to the use of an electronic nose to characterize the origin of PDO Garda EVOO. Moreover, main compounds, mainly carbonyl compounds, alcohols, esters and hydrocarbons, were found in the volatile fraction of VOO (Flath et al., 1973). These volatile compounds, stimulating the olfactive receptors, in the human nose are responsible for the whole aroma of the VOO; similarly in the electronic nose a variety of sensors interact differently with the odors of the sample. Volatile components of olive oil are considered as a key for quality and authentication control; consequently they are of a big interest. In the literature, there are several examples that demonstrate the possibility of using an electronic nose for the quality control of olive oil aroma (Guadarrama et al., 2000). The combination of electronic nose fingerprinting with multivariate analysis also provides an original approach to study the profile of olive oil in relation to its geographical origin (Ballabio et al., 2006; Cosio et al., 2006).

In the present study, EVOOs have been studied by means of an electronic nose and by classical chemical parameters.

The data set has included 53 samples of monovarietal EVOOs obtained from several olive cultivars and grown in 5 different regions: Garda, 36 samples; Spain, 6 samples; Sardinia, 5 samples; Campania, 4 samples; Abruzzo, 2 samples. The sampling has included also 19 commercial and multivarietal EVOOs: 3 samples labeled as Garda PDO, produced with cultivars allowed by the Garda Production Disciplinary; 3 samples of Garda, not labeled as PDO; and 13 samples collected on the market, produced with unknown cultivars. All these commercial samples have been used only to test the classification model.

The quality of EVOO is determined by analytical parameters: free acidity, peroxide value, $K_{232}$, $K_{270}$, and $\Delta K$, according to legislation (EEC, 1991; IOOC, 2001). All of the analyzed samples have respected the law limits and consequently can be considered EVOOs. Total phenols have also been determined for all the samples, in order to verify a possible correlation with their geographical origin. For the same reason, the oil samples have been analyzed by means of electronic nose, which could be able to detect the presence of volatile compounds in olive oils.

All data collected from the electronic nose were compared and elaborated, together with the chemical parameters. As a first step, principal component analysis (PCA) was carried out using the complete data set. Then, classification technique (LDA) was applied.

PCA was performed on the autoscaled data in order to provide partial visualization of the data set in a reduced dimension. The two principal components represent 79% of the total variance. Examining the score plot (Fig. 9.4) in the area defined by the first two principal components, a clear separation of the samples into five groups was found according to the region of origin.

In order to characterize EVOO samples into the five mentioned classes, a supervised pattern recognition method was applied. LDA applied to the complete data set gave a recognition percentage of 100% for all EVOOs (error rate 0%), while during the leave-one-out cross-validation, some samples were not correctly classified (cross-validation error rate of 7.55%).

Finally, the classification model was applied to a new set of electronic nose data, that is, the 19 commercial and multivarietal EVOOs. Fig. 9.5 shows the predictive ability of LDA model. It can be seen that all the samples came from Garda were correctly classified, whereas the multivarietal EVOOs were distributed among the other classes due probably to its characteristic of being a mixture of oils from different geographical areas.

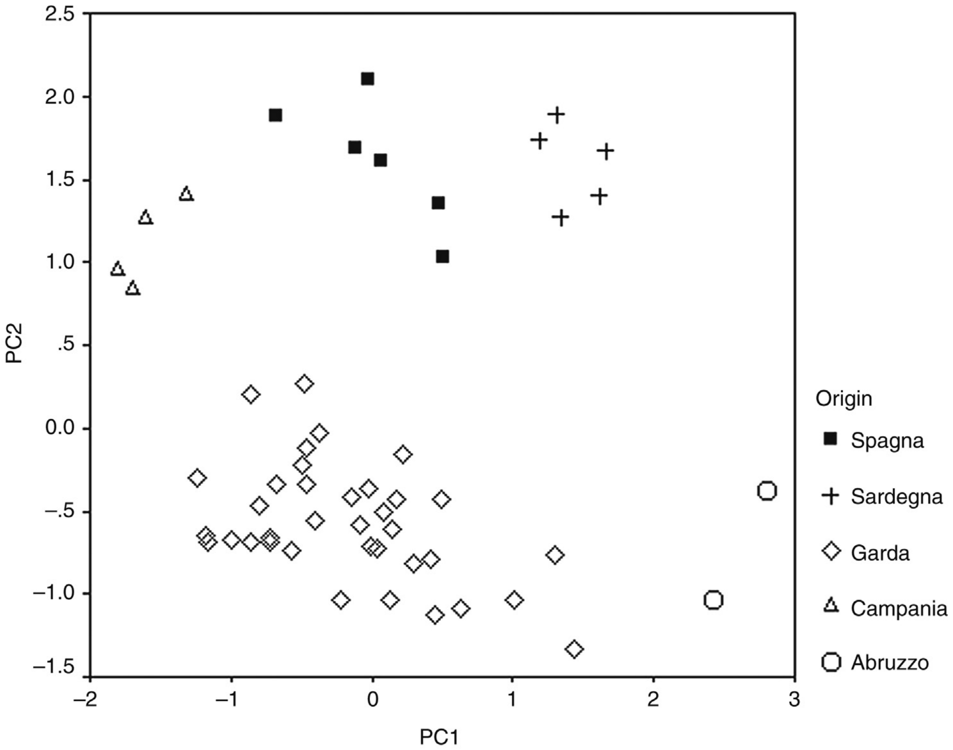

**FIGURE 9.4 PCA on autoscaled data: score plot.** Groups are shown with different symbols.

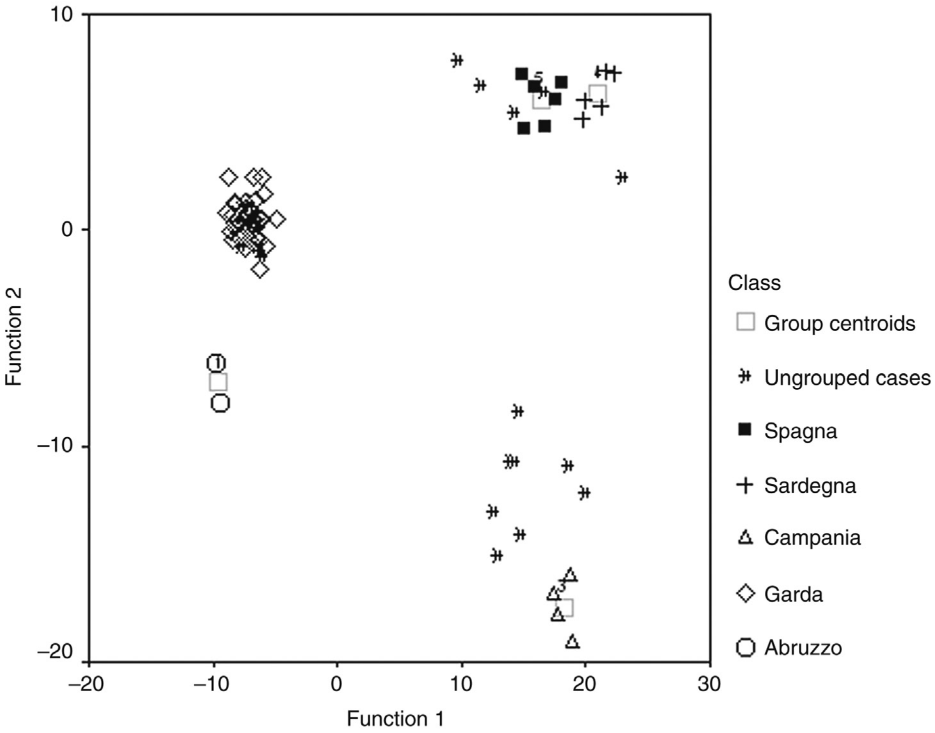

**FIGURE 9.5 Projection of monovarietal EVOOs, commercial and multivarietal EVOOs predicted by the LDA model.**

This study clearly shows that it is possible to differentiate and classify EVOOs from different geographical areas by using a commercial electronic nose and by applying multidimensional chemometric techniques.

In conclusion, there is a growing emphasis and consensus that intelligent sensor array or electronic noses are most effective in the quality control of raw and manufactured products, for example, determination of food freshness and maturity monitoring, shelf-life investigations, authenticity assessments of products, and even microbial pathogen detection and environmental control.

This application area is particularly important because the e-nose can be trained to recognize hazardous chemicals as well as odors. Furthermore, with respect to the human nose, the e-nose does not fatigue as easily, is less costly, and can easily be transported. It also holds the promise of being much cheaper, smaller, and easier to use and maintain than a mass spectrometer.

## 9.4 CASE STUDY 3: CLASSIFICATION OF EVOO SAMPLES BY PTR-MS

The discrimination capability of PTR-MS are discussed next in connection with MS-e-nose based on PTR-MS. Different olive oils were submitted to accelerate thermal oxidation and compared to their untreated counterparts. PTR-MS was able to discriminate between oxidized and nonoxidized samples, as determined by means of partial least square-discriminant analysis (PLS-DA). Employing multivariate calibration techniques, mass spectral fingerprints could also be correlated with the degree of oxidation, as expressed by the peroxide value. Based on the mass spectra of pure standards, some mass peaks correlating with the degree of oxidation could be assigned to known degradation products. This was the case with peak *m/z* 111, attributed to octanal, a known product of the oxidative breakdown of oleic acid (Aprea et al., 2006; Cappellin et al., 2013). This is a representative example of the capability of PTR-MS to generate data that go beyond the mere fingerprint. PTR-MS was also applied to the solution of more complex problems of olive oil classification, such as the determination of the country or the cultivar of origin (Araghipour et al., 2008; Ruiz-Samblás et al., 2012).

## 9.5 CASE STUDY 4: PROCESS MONITORING BY PTR-MS

As observed for traditional electronic noses, PTR-MS offers the possibility to perform real-time monitoring of reactions and processes. This is easily exemplified by an experiment (Aprea et al., 2008) where an olive oil sample was connected to the instrument inlet, gradually heated to 77°C and continuously monitored. Fig. 9.6 shows typical evolution profiles for three mass peaks, tentatively attributed to *t*-2-hexenal, hexanal, and nonanal. The former two carry herbaceous and fruity notes, while the latter is commonly linked to disagreeable *fatty*, *paint-like*, and *waxy* odors; all compounds are generated from fatty acids, either by enzymatic breakdown or by autoxidation. Fig. 9.6 shows how the signal attributed to nonanal gradually increases, while *t*-2-hexenal and hexanal show a more complex pattern. Hence, nonanal is probably formed during the accelerated thermal oxidation, while the other two carbonyls are already present in the sample; their abundance in the headspace first increases due to heating, whereas in a further stage the overall trends are the product of the progressive evaporation caused by the constant stream of air applied to the sample, together with the gradual formation, due to oxidative breakdown. The two separate reactions are represented in Fig. 9.6 in the case of hexanal by means of a continued and dashed line, respectively.

In another application (Vezzaro et al., 2011) the PTR-MS technology was applied at different stages to the off-line measurement of the olive oils obtained from different technological processes, involving the use of nitrogen-saturated

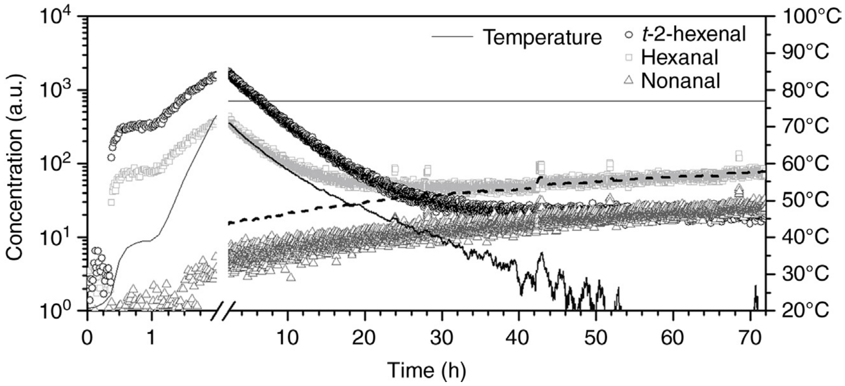

**FIGURE 9.6 Online monitoring of the thermal oxidation of olive oil, heated to 77°C.** The figure depicts the time evolution of selected *mass peaks*, tentatively attributed to volatile compounds. The *continuous* and *dashed line* represent the two separate contributes to the hexanal signal, corresponding to the reactions of formation and evaporation, respectively.

atmosphere. In this case, the analysis of mass spectral fingerprints allowed for the follow-up of the various processes. Overall, these examples demonstrate the effectiveness of PTR-MS as process analysis technique. Moreover, the data thus generated can serve to the calculation of reaction rates of oxidation reactions or partition coefficient of various VOCs.

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## Chapter 10

# Rapeseed Analysis by an Electronic Nose

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## 10.1 INTRODUCTION

Rapeseed is a source of edible oil with health-promoting nutritional properties. The increasing demand for biodiesel as a renewable energy source has boosted rapeseed oil production. This tendency was strengthened in 2007 by the European Commission's goal to increase the volume of fuels produced from renewable sources to 10% by 2020. The European Union is presently the largest rapeseed producer in the world.

The only parameter inspected in rapeseed trade turnover is the moisture content. This analysis is conducted by infrared spectrometry, which is not able to detect the presence of moldy or burnt rapeseed in the analyzed sample. Specific tests, such as the determination of ergosterol (a good indicator of mold infestation), also require special scientific equipment, such as high-performance liquid chromatography (HPLC). Species of mold developed on rapeseed produce a range of volatile organic compounds (VOCs) such as: alcohols, esters, aldehydes, ketones, terpenes, and sulfur compounds (Magan and Evans, 2000). The mixture of these chemicals creates a typical musty or moldy smell, which is the main indicator of mold infestation for humans.

In food-processing plants, rapeseed quality is most often assessed by a panel of trained experts. Nonetheless, this method is not objective and is characterized by low repeatability. Efforts are increasingly often being made to objectivize and automate this evaluation, for example, through using electronic nose technology. Rapid, robust, and efficient instrumental analysis methods for the evaluation of rapeseed quality would be of interest not only for edible oil producers, but also for farmers who could immediately and independently judge the quality of their rapeseed (Kubiak et al., 2012).

Electronic nose technology enables an objective evaluation of raw material aroma in the food industry. It has been successfully applied in the quality assessment of cocoa beans (Olunloyo et al., 2011), rice (Xu et al., 2014), corn seeds (Paolesse et al., 2006), as well as seeds of wheat, rye, barley, and oats (Börjesson et al., 1996). The classification systems used for qualitative evaluation are mainly based on the analysis of headspace composition of analyzed samples by an array of sensors. The multidimensional databases created by the sensors are used for classification based on statistical methods (Balasubramanian et al., 2007), fuzzy sets (Perrot et al., 2006), and different types of neural networks (Jonsson et al., 1997). Table 10.1 compares the various types of electronic noses used to assess seed material quality in the food industry.

The most frequently used statistical methods for electronic nose data analysis are: principal component analysis (PCA) (Xu et al., 2014; Olunloyo et al., 2011; Campagnoli et al., 2009; Falasconi et al. 2005), linear (LDA) or quadratic discriminant analysis (QDA) (Balasubramanian et al., 2007), and cluster analysis (CA) (Janzen et al., 2006). Neural networks were also used for electronic nose data analysis. These networks are capable of modeling the properties of a nonlinear system, as observed between the input and output layers. They are useful tools for food safety and quality analyses, as well as predicting physical, chemical, functional, and sensory properties of food products during processing and distribution (Huang et al., 2007). Neural networks are able to learn through experience (like people), and they have found many applications in identifying information encoded in signals generated by the electronic nose sensors. The ability to generalize (obtaining the correct network response in a situation when input data has never been used for network training before) makes neural networks powerful tools to predict the quality of examined products using an electronic nose. In most situations, the diversity of types of neural networks available, the quantity of variables, the internal structure parameters, and the teaching rules and their specificity require an individual approach to each problem.

A measuring system consisting of 15 sensors: 10 metal oxide semiconductor field effect transistor (MOSFET) sensors, four metal oxide (MOX) sensors, and one carbon dioxide ($CO_2$) sensor, were used for the microbiological evaluation of wheat, oats, barley, and rye (Jonsson et al., 1997). Gas samples were collected from the headspace of seeds heated to 50°C and then pumped into a measuring chamber.

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**TABLE 10.1** Comparison of Electronic Nose Solutions to Assess the Quality of Various Seed Materials Used in the Food Industry

| Seed Material Type | Matrix of Sensors Used in Tests | Data Analysis Method | References |
|---|---|---|---|
| Microbiological evaluation of wheat, rye, barley, and oats | 15 sensors: 10 MOSFET sensors, 4 MOX sensors, 1 $CO_2$ sensor | BPNN 15–10–*x* where *x* depending on the number of identified classes | Jonsson et al. (1997) |
| Microbiological evaluation of wheat, barley, and oats | 15 sensors: 10 MOSFET sensors, 4 MOX sensors, 1 $CO_2$ sensor | Four-layer BPNN of two different structures: 15–6–4–4 and 15–6–4–2 | Börjesson et al. (1996) |
| Corn seeds infested by fungi | 8 QMB sensors | PCA and partial least squares discriminant analysis (PLS-DA) | Paolesse et al. (2006) |
| Wheat seeds infected by *Fusarium* | 6 MOX-type sensors | PCA | Presicce et al. (2006) |
| Barley seeds infected by *Fusarium* | 32 CP sensors (Cyranose 320$^{TM}$, Cyrano Sciences, USA) | LDA and QDA | Balasubramanian et al. (2007) |
| Wheat seeds with different storage periods | 10 MOX sensors before optimization and 5 MOX sensors after optimization | PCA and two models of MLP neural networks: 10–21–5 and 5–11–5 | Zhang et al. (2007) |
| Aflatoxin in corn seed | 10 MOX sensors | PCA | Campagnoli et al. (2009) |
| Identification of rice infestation by pests | 10 MOX sensors (PEN3, Airsence Analytics GmbH, Germany) | PCA, LDA, probabilistic neural networks (PNN), and BPNN | Xu et al. (2014) |
| Cocoa beans | 6 MOX sensors | PCA and BPNN | Olunloyo et al. (2011) |
| Coffee ripening | 6 MOX sensors | PCA and *k*-NN method | Falasconi et al. (2005) |

All signals generated by 15 sensors were used as input data into a three-layer neural network consisting of 15 input neurons—10 neurons in the hidden layer and 1–4 neurons in the output layer, depending on the number of classes identified by a given network. By using a back propagation neural network (BPNN) with a 15–10–4 structure, it was possible to identify 100% of four different odors characteristic for oats (healthy, moldy, and two types of musty aroma defined as light and strong). However, such accurate identification was not achieved for samples of small amounts (from 1 to 10%) of moldy barley and moldy rye seeds mixed with healthy grains. The identification effectiveness was 60 and 80% for barley and rye, respectively. The results of identification by a neural network with a structure of 15–10–1 for wheat seed samples containing different amounts of ergosterol showed a high correlation ($R^2 = 0.88$) with the actual degree of wheat seed infection by mold.

A similar system (consisting of 15 sensors and a neural network) was applied to examine changes in the odor of wheat, barley, and oats (Börjesson et al., 1996). The neural network had the following structure: fifteen neurons in the input layer and two hidden layers consisting of six and four neurons and two or more elements in the output layer. The identification results were comparable with the identification carried out by a panel of experts, who evaluated the odor of the examined samples according to the following scale: normal, musty, moldy, acidic, sour, burnt, and extraneous. The best identification rate (nearly 90%) of the examined seed samples was reached for two classes: normal seeds and other seeds (musty, moldy, acidic, sour, burnt, and extraneous). However, the rate of correct identification of the same samples, when more classes were analyzed, did not exceed 75% (Börjesson et al., 1996).

The changes in the odor of wheat seed stored for 5 successive years was also assessed by an electronic nose equipped with a matrix consisting of 10 MOX-type sensors, of which only 5 were used for final calculations after optimization (Zhang et al., 2007). Two structures of neural networks were tested: a 5–11–5 structure, where signals generated by 5 sensors were the input vector, and a 10–21–5 structure, where the input vector consisted of signals from all 10 sensors. Better recognition results were obtained for the first network, in which the accuracy was 100% for the teaching sequence and 96% for the test sequence. For a network using all signals generated by MOX-type sensors, the accuracy reached 99% for the teaching sequence and 88% for the test sequence (Zhang et al., 2007).

Although the results presented in the quoted papers are quite good, the analyzed data did not consider other factors affecting the odor composition of seeds. The networks are completely "unprotected" against fraud, for example, by intermixing seeds originating from crops harvested in different years or when a mixture of healthy and microbiologically infected seeds (in different concentrations) is subject to assessment.

## 10.2 QUALITY ASSESSMENT CRITERIA FOR RAPESEED

Rapeseed quality assessment is generally carried out at each processing stage: during harvest, transport, and storage in elevators, until final processing of these seeds in the oil plant. Rapeseed should have proper color, be free from extraneous odors and pests, and satisfy the assumed quality requirements. Example images of rapeseeds differing in microbiological infestation extent are presented in Fig. 10.1.

The main criterion for rapeseed evaluation by a panel of experts is to identify seed samples characterized by a musty odor, a fermentation odor, or a burnt odor. A musty odor is generated as a result of developing mold and fungi. A fermentation odor is connected with the growth of bacterial microflora. A burnt odor results from an incorrect rapeseed drying process. When even slightly microbiologically infested or burnt rapeseed is released for production, the quality of produced oil will be deteriorated (Cejpek et al., 1998). In order to verify this, additional, expensive, and labor-consuming laboratory tests must be conducted using specialist test equipment, including HPLC (Mińkowski et al., 2011), gas chromatography with a mass spectrometer (GC–MS) (Cejpek et al., 1998), or UV–VIS spectrophotometers, to estimate chlorophyll dye content (Rotkiewicz et al., 2002). Moreover, the rapeseed oil contained in seeds which have been mechanically damaged is exposed to oxidation and becomes rancid, which also changes its odor. Although other chemical methods are used to determine the oxidation state of fats, including peroxide value (PV), anisidine value (AV), and TBARS (thiobarbituric acid reactive substances) (Mildner-Szkudlarz et al., 2007), a panel of experts is still the main method of rapeseed oil rancidity degree evaluation.

## 10.3 IDENTIFICATION OF MICROBIOLOGICALLY INFESTED AND BURNT RAPESEED USING AN ELECTRONIC NOSE EQUIPPED WITH CONDUCTING POLYMER SENSORS

Rapeseed with a musty and fermented odor was identified by an electronic nose and a panel of experts (Kubiak and Mikrut, 2005). The rapeseed samples (200 g) with diverse moisture content (5% for seeds with natural odor) and rapeseed samples with a moisture content ranging from 13 to 34% were analyzed. The samples were kept at a temperature of 20°C and were evaluated by a panel of experts after 24 and 72 h. The experts' task was to qualify the examined samples into one of three classes: seeds with natural odor, seeds with a musty odor, and seeds with a fermented odor. The quality of the same rapeseed samples was also assessed by a Cyranose 320 electronic nose (Cyrano Science, Pasadena, California, USA) equipped with 32 conducting polymer (CP) sensors. The polymer sensors were nonspecific to any particular gas, but each sensor had a certain degree of affinity toward specific chemical or volatile compounds (Balasubramanian et al., 2007). The sensor responses obtained as a 32-sensor matrix were used for further analysis carried out with neural networks and the *k*-nearest neighbor (*k*-NN) method. The neural networks were implemented with PRTools software (Duin, 2000). The output layer of the network consisted of three neurons corresponding to the number of classes, which were identified by a given network (rapeseed: healthy, musty, and fermented) and the number of elements in the hidden layer changed from 3 to 30. Three different sequences were used in the computations, consisting of the sensory spectra obtained for rapeseed with different moisture contents and

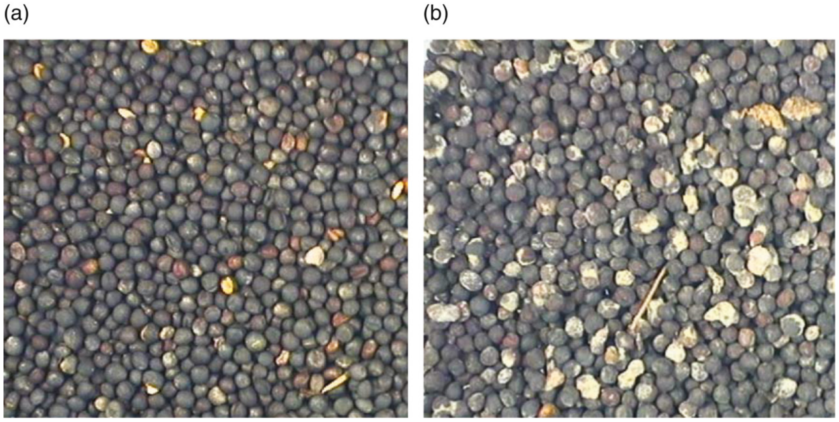

**FIGURE 10.1 Images of rapeseed samples differing in the extent of microbiological infestation.** (a) Healthy seeds with natural odor and (b) rapeseeds with musty odor with visible seed infestation by mold.

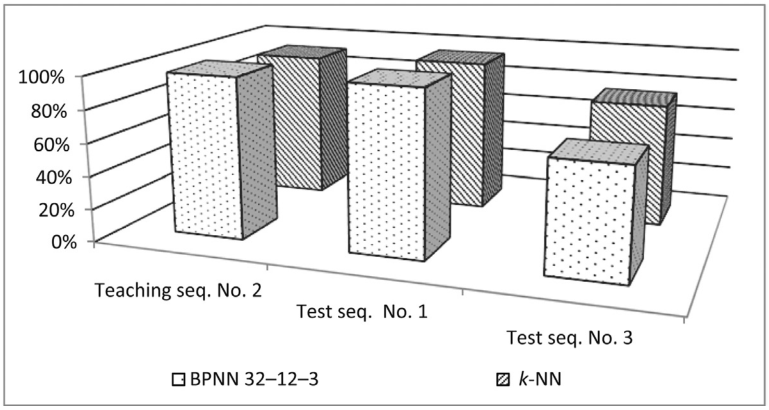

**FIGURE 10.2 Comparison of identification results for the teaching sequence and two test sequences obtained for the optimal BPNN 32–12–3 and the *k*-NN method.**

storage times. The computations carried out for each of these three successive sequences were used as the teaching sequence. Each time, the other two sequences were used as the test sequences. The best identification results (Fig. 10.2) for odor samples were reached for the neural network with a structure of 32–12–3, taught by a set formed on the basis of rapeseed odor spectra for seeds with diverse moisture content, obtained after 72 h of storage. The network successfully identified rapeseed samples stored for a shorter time, for example, after 24 h, as well as rapeseed samples with odor determined by rapeseed with a moisture content other than the samples used to build the teaching sequence. Identification accuracy for the three types of seed odors: natural, musty, and fermented, obtained by the neural network for the test sequence of the same moisture content was 100%, and only 67% for the test sequence, in which seeds with higher moisture contents were used. Only once the neural network identified seeds with a natural odor as seeds with a musty odor. In all other cases, erroneous recognitions concerned odors of musty or fermented seeds. Rapeseed odor identification for the same data using the *k*-nearest neighbor method was 92% for the teaching sequence and 94 and 75% for test sequences No. 1 and No. 3, respectively (Fig. 10.2).

The moisture content of rapeseed depends on the weather conditions, the date harvested, and the harvest method. The moisture content of rapeseed harvested at an optimal time and in good weather conditions should not exceed 9%, whereas the moisture content of rapeseed harvested during heavy rain may even exceed 20%. A high rapeseed moisture content requires almost immediate drying. If this process is carried out at the wrong drying parameters (too high drying air temperature, incorrect flow rate, and duration of material kept in the drying zone) or using a dryer of obsolete design, the dried material is exposed to the risk of burning. Burnt rapeseed is useless for processing purposes and adding even a small amount of burnt rapeseed to healthy rapeseed may result in a deteriorated oil quality and an increase in the presence of potentially carcinogenic substances, such as polycyclic aromatic hydrocarbons (PAHs) in crude oil (Cejpek et al., 1998).

The problem of identifying the following seed types: microbiologically infested seeds, burnt seeds, and burnt seeds mixed with healthy seeds, was discussed in another publication (Kubiak and Mikrut, 2006). The authors used the sensory spectra of 32 conducting polymers (Cyranose 320). The material used in this research study consisted of samples of rapeseed with varying moisture contents, ranging from 5% for the reference sample (natural smell), 13% for a rapeseed sample with a moldy smell, to 34% for rapeseed samples exhibiting a fermented smell. Additionally, burnt rapeseed samples of varying composition, starting from 100, 10, and 5% down to 1%, were analyzed. Two types of software for neural network implementation were used for data analysis using the NeuralWorks Professional II/Plus version 5.52 software package (NeuralWare, Pasadena, United States of America) and the PRTools software package. Additionally, in order to compare the obtained results with the classical method, another option of PRTools was also employed, which allowed the recognition of the same data using the *k*-NN method. In the calculations, four different data sets were used with different representation of each type of smell: natural, moldy, fermented, and burnt. The proper selection of data representation can play a key role in the recognition of burnt rapeseed samples (Kubiak and Mikrut, 2006). When a set with 10% burnt rapeseed was applied as a learning set for the neural network using NeuralWorks Professional II/Plus version 5.52 software, the recognition rate of the rapeseed smell by the network (with the number of elements in the hidden layer varying between 3 and 30) averaged about 93%. The recognition accuracy for a set with 100% burnt rapeseed used as a test set averaged 74%. The majority of the recognition errors in the test set were caused by the erroneous recognition of the burnt smell (100%) as a moldy smell. However, when a set with 100% burnt rapeseed was applied as a learning set, the recognition rate for a test set with 10% burnt rapeseed averaged 71%. For this combination of sample sets, most of

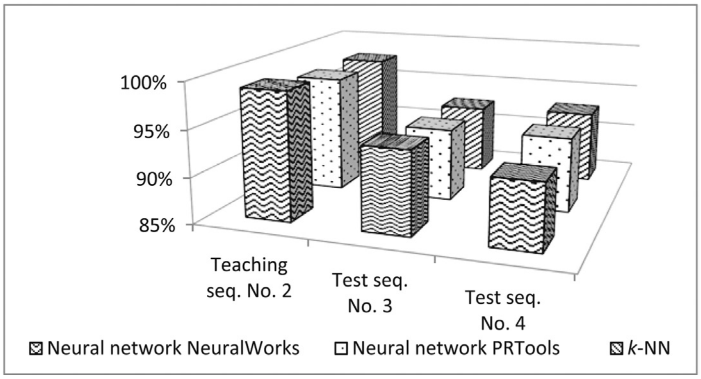

**FIGURE 10.3 Comparison of identification results for the teaching sequence and two test sequences obtained for the optimal neural network calculated with NeuralWare and PRTools software as well as the *k*-NN method.**

the recognition errors in the test set were caused by the erroneous recognition of burnt rapeseed (10% concentration) as seeds with a natural smell. A similar tendency with a lower level of average recognition was observed for the same data sets implemented in the PRTools software package for neural network and *k*-NN calculations. Poor recognition results for the burnt rapeseed samples forced Kubiak and Mikrut (2006) to increase the representation of burnt rapeseed sample in the teaching set (No. 2). A modified teaching set was used in the next stage of the calculations. The representation of the burnt rapeseed was increased by an additional 15 spectra, collected for burnt rapeseed with a 100% concentration. To test the new neural network, the two new sets were applied using burnt rapeseed percentages of 5% (No. 3) and 1% (No. 4). Fig. 10.3 presents a comparison of the identification results for the teaching sequence and two test sequences obtained for the optimal neural network calculated with NeuralWorks software, PRTools software, and the *k*-nearest neighbor method. The recognition rate for the neural network calculated with the NeuralWorks software reached 97% for the test set with a 5% representation of burnt rapeseed (test set No. 3), which was slightly worse than the recognition rate for test set No. 4 (98%) in which the representation of burnt seeds was 1%. The average recognition reliability achieved for the neural networks implemented in the PRTools software was 93% for both test sets (No. 3 and No. 4). The average recognition rate achieved by the *k*-nearest neighbor method was 93% for test set No. 3 and 92% for test set No. 4.

## 10.4 IDENTIFICATION OF MICROBIOLOGICALLY INFESTED AND BURNT RAPESEED USING AN ELECTRONIC NOSE EQUIPPED WITH METAL OXIDE AND QUARTZ MICROBALANCE SENSORS

One of the challenging problems of rapeseed commodity turnover is the detection of small amounts (below 10%) of moldy or burnt rapeseed mixed with the healthy seeds. This situation may be inadvertently created by the mixing of sound rapeseed with burnt, musty, or moldy rapeseed during transport and its distribution into silos for storage. Attempted fraud by diluting burnt or moldy rapeseed with sound rapeseed should also be considered (Kubiak et al., 2012). Due to rapeseed production process diversification in Europe, the detection of relatively small amounts of rapeseed contamination may be additionally obstructed as a result of odor differences between individual rapeseed varieties and different cultivation areas. These differences are primarily connected with agrotechnical practices during rapeseed cultivation (the volume and type of fertilizers and pesticides applied), harvest time, and rapeseed maturity. Additionally, plant genetics and plant hormones strongly influence the biosynthesis pathways responsible for the release of volatile compounds (Baietto and Wilson, 2015).

The aroma changes caused by adding a small amount of mold or burnt rapeseed in four rapeseed varieties harvested during the same season from two different locations (three varieties in Poland and one variety in Belgium) were investigated (Kubiak et al., 2012). Samples with different levels of moldy rapeseed were specially prepared to reach 1, 3, 5, and 10% moldy seed concentrations in sound rapeseed. A similar procedure was applied to samples of burnt rapeseed to reach the following levels of concentration: 1, 3, 5, and 10%. For aroma evaluation of the contaminated samples, the instrument setup consisted of a VOCmeter electronic nose (AppliedSensor GmbH, Reutlingen, Germany) connected to a headspace sampler G1883 (Agilent Technologies, Cernusco, Italy) equipped with four MOX and eight quartz microbalance sensors (QMB). The results of the classification of all rapeseed samples with different concentrations of moldy seeds were analyzed by discriminant function analysis (DFA). DFA performed on the data of individual rapeseed varieties allowed a clear discrimination of sound rapeseed samples from both samples containing different levels of moldy or burnt rapeseed. However, the increased variability introduced into the merged data sets resulting from

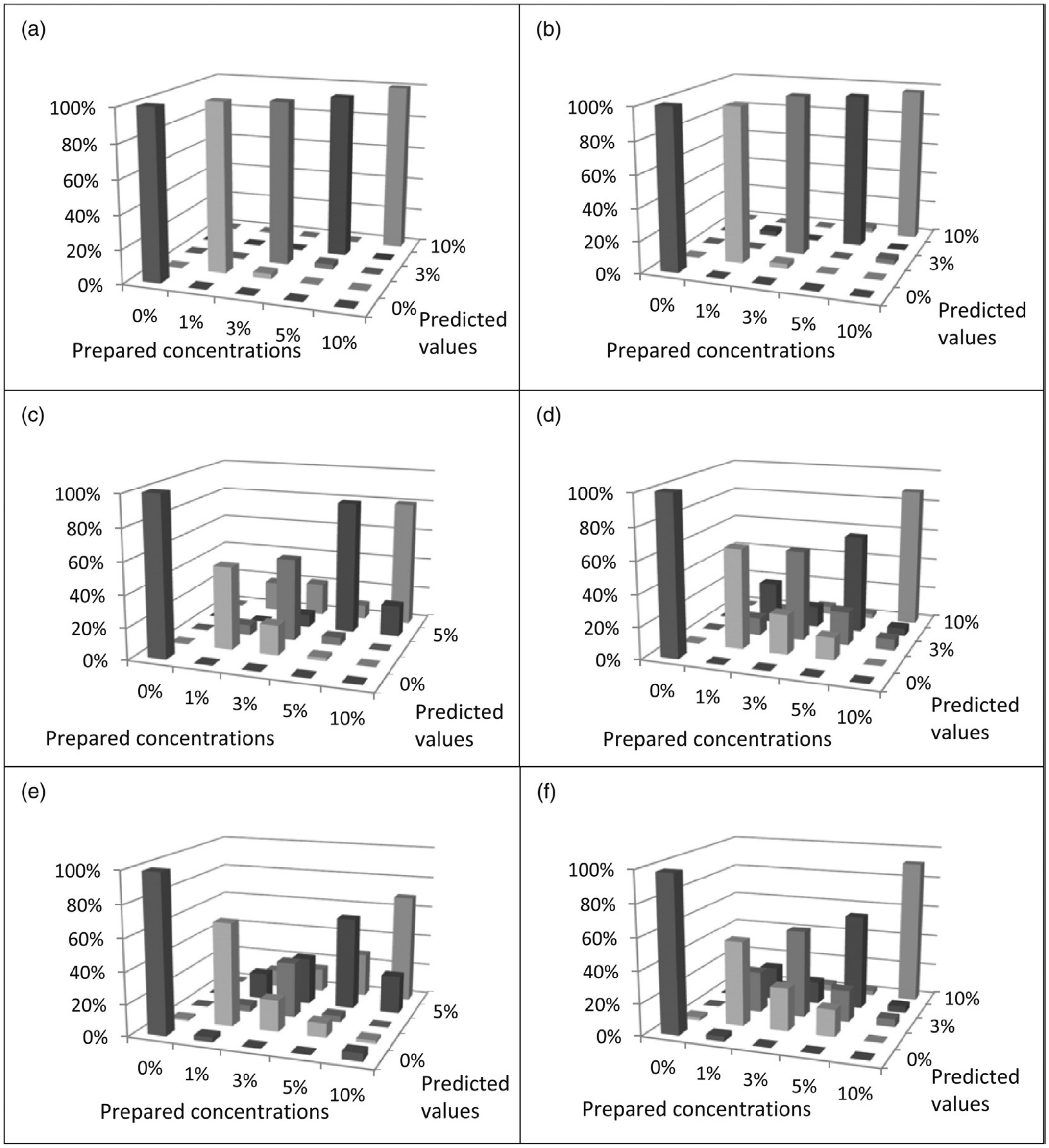

**FIGURE 10.4 Comparison of identification results obtained by DFA classification.** (a) Single Polish rapeseed varieties with a small amount of moldy rapeseed; (b) single Polish rapeseed varieties with a small amount of burnt rapeseed; (c) three Polish rapeseed varieties with a small amount of moldy rapeseed; (d) three Polish rapeseed varieties with a small amount of burnt rapeseed; (e) four rapeseed varieties (three Polish and one Belgian) with a small amount of mold rapeseed; and (f) four rapeseed varieties (three Polish and one Belgian) with a small amount of burnt rapeseed.

differences in the varieties of Polish rapeseed and geographical and climatic conditions (by applying the Belgian rapeseed variety) caused more ambiguity in the results of the data evaluation (Fig. 10.4). A low percentage of sound samples were misclassified, leading to false positive assignments and a few faulty samples were assigned to the class of sound rapeseed samples. Misclassifications occurred predominantly among the different contamination levels. For quick and impartial evaluation of rapeseed quality by an electronic nose, a "threshold" analysis could be a solution (Kubiak et al., 2012). The use of the threshold method is based on the assumption that, as a practical matter, it is more important to identify faulty rapeseed and to separate it from sound rapeseed than to identify the particular type of deficiency. The total percentage of samples correctly classified in the experiments (both sound and faulty, including moldy rapeseed at all contamination levels) exceeded 98.6%. Even more favorable was the threshold analysis of burnt rapeseed samples, where none of the investigated samples were misclassified, indicating the high potential of electronic nose technology to evaluate rapeseed quality (Kubiak et al., 2012).

## 10.5 IDENTIFICATION OF MICROBIOLOGICALLY INFESTED RAPESEED CARRIED OUT USING AN ELECTRONIC NOSE EQUIPPED WITH COLORIMETRIC ODOR SENSORS

Colorimetric odor microsensors are matrixes of several dozen metalloporphyrin sensors applied onto a hydrophobic membrane and closed in a tight measuring capsule. Each sensor has different characteristics, which makes them react differently to volatile compounds in the tested gas sample. The main advantage of colorimetric odor microsensors is that by analyzing the color changes of each sensor, the odor profiles of the tested compounds can be developed (Kubiak, 2014). Colorimetric odor sensors were used in medicine (Mazzone et al., 2007), chemistry (Janzen et al., 2006; Bang et al., 2008), and food processing (Zhang et al., 2006; Zhang and Suslick, 2007; Lim et al., 2008). The methodology of analyzing changes in microsensor color used in the majority of the publications is based on analyzing the differences in pictures taken before and after a microsensor array is exposed to an analyte. Subsequently, the average values for the color components (R, G, B) for each sensor were calculated and a 3*N*-dimensional vector was created (where *N* is the number of colorimetric microsensors), which showed the extent of the color changes of all the sensors. The quantities were subsequently analyzed with standard statistical techniques, such as PCA (Suslick et al., 2004) or CA (Janzen et al., 2006). Colorimetric sensor arrays were also used for rapeseed quality evaluation (Kubiak, 2014). Examples of differential images of colorimetric sensor arrays recorded during the first 24 h for the rapeseed sample with a natural odor are presented in Fig. 10.5.

The changes in the color of colorimetric odor sensors recorded during the first 5 days of the experiment for rapeseed with an elevated water content from 7 to 16% are presented in Fig. 10.6.

Due to the strongly nonlinear nature of the registered changes in individual RGB color components, the results obtained were analyzed using the BPNN. For data analysis, the "threshold" analysis was applied in which the values obtained from the output layer of neural network were compared with a certain threshold value (Kubiak, 2014). When the value of the neural response was higher than the threshold value, it was regarded as recognition, else it was regarded as an absence of recognition.

An analysis of the results (Kubiak, 2014) obtained for the training set shows that 96% recognition of healthy rapeseed and 100% recognition of musty rapeseed can be achieved at the threshold value of 0.4. Such good results were achieved with a relatively low level of rejections (2%). It was found that for the same threshold value in the test sequence, the network recognized 100% of healthy seeds and 98% of infested seeds. For the threshold of 0.5, the network recognized 100% of healthy and 100% of musty (microbiologically affected) rapeseeds. The level of no

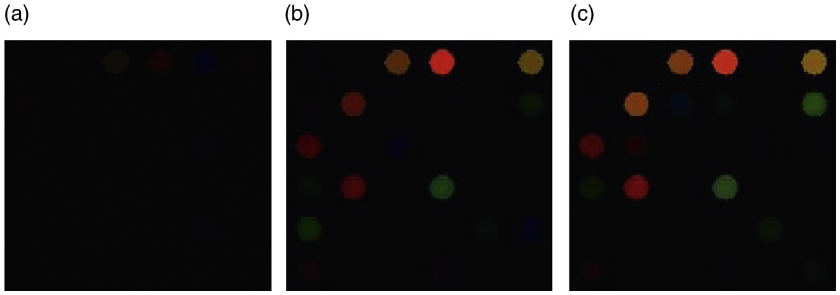

**FIGURE 10.5 Examples of differential images recorded for a sample with an odor typical of healthy rapeseed (water content, 7%).** (a) 0–5 min; (b) 0–1 h; and (c) 0–24 h.

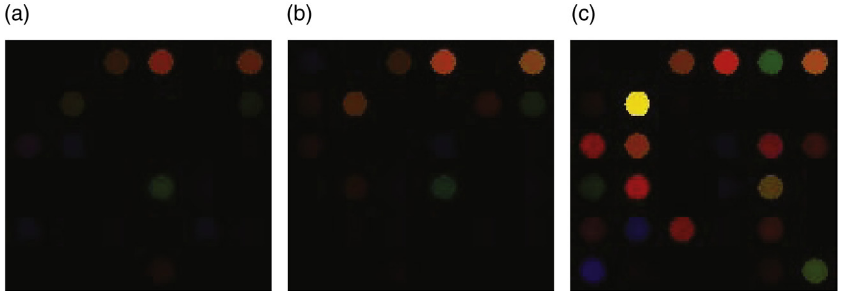

**FIGURE 10.6 Examples of differential images recorded during the first 120 h of the experiment for rapeseed samples with a water content of 16%.** (a) 0–1 h; (b) 0–16 h; and (c) 0–120 h.

recognition (rejections) for these threshold values was 5% for both the teaching sequence and the test sequence.

Since three rapeseed cultivars with different moisture contents were studied, which could also result in different levels of infestation, the 94% recognition effectiveness in the test sequence for rapeseed with a characteristic healthy odor and 98% for a musty odor may be regarded as satisfactory. With an additional criterion of the threshold analysis, it is even possible to increase the level of recognition to 100% for rapeseed with a healthy or musty odor. Thanks to the application of the threshold analysis, it was possible to determine the threshold value necessary to obtain 100% recognition combined with information on the number of rejected samples. Additionally, knowing the threshold value, data on the recognition confidence can be evaluated. This approach of data analysis allows for the utilization of the electronic nose in industrial applications for rapeseed quality evaluation.

## 10.6 SUMMARY AND PROSPECTS

Signals generated by the matrix of electronic nose sensors require extraction of uncorrelated features of the examined signals and choosing an appropriate strategy for their selection. In most publications, this is done through trial and error, and only in some cases are all features systematically searched through in order to eliminate excess representation and obtain optimal results. In most studies presented in literature, rapeseed quality was assessed in a static manner, involving the need to analyze a considerable number of samples and to apply an appropriate algorithm for sample taking. The selection of an appropriate method, types, and structures of neural networks (and network teaching and testing algorithms) fully allows for their use when assessing the quality of grainy materials. Very often, the level of correct recognition of an examined grainy material ranged between 80 and 98%, which is a very good result for such inhomogeneous material as grain cereal seeds. It is hard to expect results reaching 100% because it is impossible for a random sample (minimum several hundred seeds) to have properties comparable to the data used for network teaching. The material itself can also vary, depending on its variety and the agrotechnical conditions it was cultivated under, which should be the subject of further research.

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Chapter 11

# Rice and the Electronic Nose

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## 11.1 INTRODUCTION

Rice (*Oryza sativa* L.) is one of the most important staple food crops in the world other than wheat and corn. More than half of the world's population depends on rice for their living, particularly in Southeast Asia, China, and India (Dorosh and Wailes, 2010). Several categories of rice include brown rice, long grain, medium/short grain, parboiled rice, aromatic rice, and glutinous rice. Brown rice is produced by removing the outer husks, while white rice is processed by removing the whole husk.

Amylose is an important starch in rice for energy storage, which is more resistant to digestion. Long-grain rice that contains more amylose is considered to be high-quality rice because of its physical appearance, and it contributes about 75% of the world's rice market (Wailes, 2005). Whereas the medium/short-grain rice contains less amylose and accounts for only 12% of the market. However, brown rice contains more nutritional components, such as dietary fibers, phatic acids, and vitamin B and E than white rice (Champagne et al., 2004). Aromatic rice variety is usually preferred by consumers for its good quality that includes fineness, shape, color, aroma, taste, and flavor (Choudhury et al., 2001). Usually consumers prefer to choose aromatic rice for festival and special occasions due to its high demand and good quality. The glutinous rice contains low amylose, making it sticky once cooked and is very popular in Asia.

## 11.2 QUALITY OF RICE

The quality of rice is influenced by various factors such as cultivated location, climatic conditions, genetics, and postharvest activities (Champagne, 2008). The quality characteristic is judged by the physical appearance such as size, shape, color, and cleanliness that determine the market grade (Bergman et al., 2004). The milling and processing that determine the percentage of moisture content, broken rice, and foreign materials also influence the grade of rice (Shi et al., 2000). Some other quality attributes include rice cooking texture, aroma, taste, flavor, brightness, amylase content, and nutritional content (Manawthukha, 2005). Other features such as rice fat acidity characteristics are also considered because a high level of rice fat acidity will reduce the market demand (Lam and Proctor, 2003).

Rice is cultivated geographically between 50 and 35 degree latitude in various locations of the world, from lowland to hill top (Swaminathan, 1999). Basically, rice plants require a high temperature (25°C) during daytime and a low temperature (21°C) at night during the ripening stage for good aroma and kernel development (Dela Cruz et al., 1989). Rice variety selection is dependent on the area in which the crop grows from extremely wet conditions, to conditions with salinity to conditions of droughts and deserts (Swaminathan, 1999). Yoshihashi (2004) claim that intensified cultivation for stable rice production needs good irrigation systems, a consistent labor supply, and effective soil fertilizer.

Postharvest activities that influence the rice quality include the storage condition and duration, the drying technique, the enrichment process, and packaging material (Wongpornchai et al., 2004). The storage condition, time, temperature, and humidity should be controlled to minimize the effect on the rice quality such as flavor and aroma (Wongpornchai et al., 2004). The rice development programs through the cultivation and selection consider the nature of genotype, genetic, and local agroecological conditions for improvement of plant cultivar characteristics and its scale (Rahman et al., 2007). The programs study the different morphology and metabolic activity in the growing stage to enhance rice quality and yield ability. The process will select cultivars that possess good aroma, taste, and physical appearance (size, shape, and color) that are preferred by consumers (Choudhury et al., 2001).

Existing yet demanding issues in the rice industry are quality control, mislabeling, grading, and adulteration of different types of rice. For these reasons, the industry is

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currently using standard grades based on the market criteria to identify the grain. Several stages of inspections may take place during handling operations to identify the grain type and quality. Hence, it is more difficult and complicated to inspect the grain varietals' purity than other factors, such as aroma, flavor, size, color, and cleanliness. In order to overcome the predicament, a nondestructive and faster technique for rice assessment and easy handling is required.

## 11.3 CLASSIFICATION METHOD OF RICE

### 11.3.1 Nondestructive Testing

Nondestructive testing (NDT) is a system that has no physical contact with rice sample. Several NDT techniques are used to classify rice, namely nuclear magnetic resonance (NMR), machine vision**,** and acoustic and electronic nose. The NMR technique uses electromagnetic waves for rice sample quantitative analysis, while the machine vision method uses images to identify the rice varieties based on the silhouette image and color. The NMR method that uses the field strength and acoustic technique exploits the vibration properties to determine the quality of rice sample, whereas the electronic nose technique can be applied to identify different types of rice based on their sample odor or specifically known as volatile compounds.

### 11.3.2 Human Sensory Panel

The human sensory panel method performs rice quality assessment based on the aroma characteristic and is commonly employed due to strong consumer preferences (Fitzgerald and Hall, 2008). Normally rice is consumed without any seasoning, which makes the original sensory properties important for classification purposes (Champagne, 2008). Usually, the panels will inspect, inhale the sample rice aroma, and/or taste the cooked rice during the classification process. In order to perform effectively, the panel needs to attend proper training as well as comply with detailed and lengthy procedures. The panels are prone to fatigue, especially when the number of samples increases, and the findings are sometimes inconsistent (Pearce et al., 2003). Furthermore, the human aging process and illness may also affect human sensory panel assessment performance.

### 11.3.3 Chemical Analysis

The Fourier transform infrared method is used to identify the rice sample functional group. Most of the aromatic molecules have between one or two polar functional groups because molecules with more polar functional groups are generally not volatile (Strike et al., 1999).

The compounds are mainly characterized by their chemical structure and constituent functional groups, such as heterocyclic systems, double bonds, and aromatic rings. These characteristics contribute to the overall shape of the molecule that produces a specific aroma or flavor (Gardner and Bartlett, 1999). They often have delocalized conjugated electron structure typical of the benzene ring. However, many other compounds of unknown chemical structure are generically referred to as being aromatic because of their volatile nature or particular aromas and flavors (Wilson et al., 2001).

The analysis of rice's volatile compounds can be performed using gas chromatography–mass spectrometry (GC–MS) method. There are over 300 volatile compounds being identified from various aromatic and nonaromatic rice varieties (Widjaja et al., 1996). The analysis identifies the main volatile compound of rice as the 2-acetyl-1-pyrroline (2AP), which acts as the biomarker (Zeng et al., 2008). The 2AP biochemical pathway was identified as the gene for the aromatic of the rice varieties (Chen et al., 2008). The biomarker is detected in raw and cooked rice and is associated with the unique popcorn-like aroma. Other compounds are lipid oxidation products, such as hexanal, octanal, and pentane. The presence of these compounds in certain quantity will decrease the rice aromatic property and have a negative impact on consumer acceptance (Monscoor and Proctorc, 2004; Zeng et al., 2008; Champagne, 2008). Good rice cultivar should have high levels of 2AP and low levels of lipid oxidation compounds.

However, this method requires a detailed procedure and is costly (Monscoor and Proctorc, 2004). The method identifies and quantifies the sample rice volatile compounds and is therefore inappropriate for practical daily applications (Zheng et al., 2009).

### 11.3.4 Other Methods

The polymerase chain reaction (PCR) method uses microsatellite markers for the rice sample analysis. The restriction fragment length polymorphism (RFLP) method needs precise probes for the target deoxyribonucleic acid (DNA) systems to identify genetic markers in rice cultivar. The randomly amplified polymorphic DNA (RAPD) markers method allows the identification of genomic variation without prior knowledge of DNA fingerprint to reveal the differences among rice cultivar. The DNA molecular marker provides information to decide the distinctiveness of varieties and their ranking according to the number of close relatives and phylogenetic position.

However, most of these methods are quite costly, require detailed procedures, and are generally difficult to assess the rice sample accordingly. Consequently, the rice industries are in need of robust methods with good reproducibility and precision. The portable testing instrument will become the development trend of technology to overcome this problem.

**TABLE 11.1** Research on the Application of Electronic Nose and Rice

| Research Title | Authors |
|---|---|
| Discrimination of two types of basmati rice | Li (2000) |
| Analysis of the tastes of brown rice and milled rice with different milling yields using a taste sensing system | Tran et al. (2004) |
| Rapid identification of rice samples using an electronic nose | Zheng et al. (2009) |
| Embedded E-nose application to sense the food grain storage condition | Deshpande and Shaligram (2010) |
| An electronic nose system for aromatic rice classification | Abdullah (2011) |
| Regression model on electronic nose data from aromatic rice samples | Jana et al. (2012) |
| Research on the application of electronic nose in discriminating rice varieties | Huichun et al. (2013) |
| Optical electronic nose based on porphyrin and phthalocyanine thin films for rice flavor classification | Palasuek et al. (2014) |
| Mass spectrometry-based electric nose system for assessing rice quality during storage at different temperatures | Sung et al. (2014) |
| Correlation between physicochemical properties and eating qualities of rice | Ke-Xin et al. (2014) |
| Identification of grain mildewing with ANN pattern recognition software based on VB and MATLAB | Zhao et al. (2008) |
| Rapid identification of rice samples using an electronic nose | Zheng et al. (2009) |
| Identification of early moldy rice samples by PCA and PNN | Wu et al. (2012) |
| Paddy and maize moldy status characterization using electronic nose | Wang et al. (2014) |
| Discrimination of plant volatile signatures by an electronic nose: a potential technology for plant pest and disease monitoring | Laothawornkitkul et al. (2008) |
| Discrimination of different types of damage in rice plants by electronic nose | Zhou and Wang (2011) |

## 11.4 ELECTRONIC NOSE FOR RICE

Electronic nose (e-nose) is a nondestructive intelligent electronic sensing instrument, which mimics the human olfactory system to detect, discriminate, and classify odor samples (Leffingwell, 2002). The instrument is suitable for a NDT technique that produces a qualitative output. The rapid development of the instrument has been driven by its potential applications, including food quality assurance, fragrance identification, environmental monitoring, and plant disease control (Pearce et al., 2003; Mahmoudi, 2009). The instrument basically consists of the sensing chamber, array of sensors, signal processing, and pattern recognition. The sensor chamber will accommodate the array of sensors to interact in a control environment with the odor samples. The array of sensors will translate the changes in electrochemistry interaction with the samples into electrical signals such as current or voltage. When the sensor is exposed to a sample odor, it will induce a reversible physical or chemical change in the sensing material and cause a change in the output (Amamcharla, 2008). The signal conditioning will acquire the sensor responses; then isolate, amplify, and compensate the signal for linearization; and then eliminate noise or drift effect (Pearce et al., 2003). The resulting output signals are being processed using pattern recognition methods to discriminate or classify rice odor samples. The instrument is sensitive and fast in response to the different rice aroma (Patrycja and Wojciech, 2006). This will minimize the exposure to potentially harmful grain dusts, especially during in situ measurement (Cheaupun et al., 2003). The technique is appropriate to complement the human sensory panel for rice odor assessment applications. The e-nose will overcome some of the limitations of human sensory panel testing for being fast, reliable, and consistent in quality classification of grains (Zheng et al., 2009). This includes assessment in the discrimination and classification of rice samples, and identification of rice mold contamination and rice plant disease as shown in Table 11.1.

### 11.4.1 Classifying the Rice Sample

The e-nose was used for the inspection and screening of rice samples quality (Li, 2000; Ke-xin et al., 2014; Sung et al., 2014). Tran et al. (2004) analyzed the tastes (e-nose or tongue) for brown and milled rice of several milling yields and were able to differentiate between raw and cooked samples. The instrument was used as a rapid technique in differentiating rice samples (Zheng et al., 2009). An embedded sensor system was included in the instrument to study the deterioration of grains under different stress by performing different analysis (Deshpande and Shaligram, 2010). The developed instrument was then used for aromatic rice classification (Abdullah, 2011; Jana et al., 2012). The instrument was also used for rice odor evaluation to distinguish different rice varieties and species (Huichun et al., 2013; Palasuek et al., 2014).

### 11.4.2 Rice Pests and Mold Contamination

Rice mold could produce toxic substances, which is unsuitable for human consumption as it can cause allergic reactions and diseases. So a fast, reliable, real-time analysis method for rice mold status monitoring is required (Wang et al., 2014). The e-nose was used to detect the pests in the rice samples by acquiring the odor using headspace sampling. Then pattern recognition was used to classify the different rice sample mold status. The technique minimized the exposure to toxic substances in rice during the sampling process. Mildewed rice was assessed by using a smell autoanalysis system. (Zhao et al., 2008). The instrument was used to differentiate between damaged and undamaged rice by analyzing the odor sample (Zheng et al., 2009). Pattern recognition methods were effectively used to classify different degrees of moldy rice in the early stage by using different levels of mildew data on rice samples (Wu et al., 2012). The instrument was also used to distinguish paddy and maize samples for different mold status. The pattern recognition model was able to predict the samples with a high accuracy rate (Wang et al., 2014).

### 11.4.3 Rice Plant Disease

Rice plant diseases are caused by microbes that will decrease the crop yield. Rice plants would produce pest-induced volatile compounds, which the e-nose can be used for plant insect monitoring (Laothawornkitkul et al., 2008). Pests like the brown plant-hoppers can cause infestation that would damage rice plants. The instrument was used to discriminate the different volatile profiles emitted by uninfested rice plants (Zhou and Wang, 2011).

## 11.5 DATA ANALYSIS

The electronic nose array sensors produce a specific multivariate time-series response. The nature of data depends on several factors such as ambient volatile compounds, temperature, and humidity. The relevant chemical information from the sample data will be analyzed using a chemometric technique that applies mathematical, statistical, and graphical approaches. These pattern recognition techniques use multivariate analysis to discriminate data from the instrument's array of sensors simultaneously. The process uses either a statistical or intelligent classification technique to recognize, distinguish, and classify the odor sample data qualitatively or quasiquantitatively, which is vital for the instrument.

Before these data can be analyzed, a preprocessing technique is applied. The preprocessing of data will enhance the quality of sensor responses by removing redundant and irrelevant information. The process also filters outliers and compensates noise and drift variation, while retaining much of important data (Arshak et al., 2003). The process includes feature selection, baseline manipulation, and normalization to make it appropriate for the pattern recognition analysis.

The sensor response is time-dependent data. They consist of the initial dynamic slope response or transient responses and the steady-state response for both sniffing and purging processes. Feature selection selects a certain region of the sensor responses that contain relevant information for the pattern recognition. Most of the developed electronic noses are used to measure static or steady-state responses to extract the features of the sample response (Lozano et al., 2008). Recent studies have shown that the use of both transient and steady-state signals are better than using exclusively steady-state signal (Trincavelli et al., 2009). However, the transient signal techniques include the amplitude, curve fitting, fast Fourier transform (FFT) coefficients and the wavelet transform, which needs a longer time to achieve a sample recognition rate compared with steady-state signals (Szczurek and Maciejewska, 2013).

The baseline manipulation is a data manipulation technique based on the difference between reference or background and sample sensor responses. This option reduces the dominance of sensors that have high response levels, which minimize the effect of temperature, humidity, and temporal drifts (Gardner and Bartlett, 1999). The reference normally is the ambient air or a gas such as nitrogen. The baseline manipulation data that contains the relevant information will enhance the instrument performance.

The normalization technique is used to rescale the sensor responses to make them fit for pattern recognition processing. The option reduces the effects of sensory dominance, sample concentration, and an outlier. This enhances the raw data, which will reduce the computational errors (Gardner and Bartlett, 1994).

Finally, before the analysis is performed, data shall be checked for normality. The normality test is used to investigate whether data is normally distributed (parametric) or not normally distributed (nonparametric). Among the available tests include Kolmogorov–Smirnov, Lilliefor, and Jarque–Bera methods (Razali and Wah, 2011). The data or features distribution are also being used in the pattern ruling, especially when outliers are present (González, 2007). The use of the correct classification technique according to its correct nature is to ensure the validation of the result.

### 11.5.1 Discrimination and Classification of Rice

There are several multivariate data analysis techniques for discrimination and classification, which can be categorized into supervised versus unsupervised, or, parametric or nonparametric. For this analysis, focus is given to the supervised and nonparametric approaches which include the *k*-nearest neighbors (*k*-NN), support vector machines (SVM), multilayer perceptron (MLP), and radial basis function (RBF). The *k*-NN is a technique for classifying samples based on the closest distance to the training model. The simple machine learning algorithms are based on the distance between the unknown sample and the training model. Squared Euclidean has been chosen for distance measurement since it gives a better performance even if the number of *k* is small (Bonet et al., 2008).

SVM is used to develop the individual hypothesis. SVM is a popular algorithm applied in the learning machine. It can be used for classification, regression, and other learning tasks (Chih-Chung and Chih-Jen, 2011). The technique is capable of learning high-dimensional space with few training data (Chih-Chung and Chih-Jen, 2011). The basic concept of SVM is to search for an optimal separation in the hyperplane, where it can separate instances into two classes.

MLP is the most popular type of supervised artificial neural network (ANN) due to its simplicity and performance (Svozil et al., 1997). A typical structure has three layers; one input layer, one hidden layer, and one output layer. Each layer is fully connected to the earlier layer and has no other connection (Nelson and Illingworth, 1991). The input layer is connected with each of the instrument sensors. The activation function is used to transform the result of limiting the permissible amplitude to some finite value and pass it to the next layer. Some of the popular activation functions are the linear function, the hyperbolic tangent activation function, and the sigmoid activation function.

RBF is a type of supervised ANN that uses transmission of information in a forward approach. The architecture consists of one input layer, one hidden layer, and one output layer. The technique approximation capabilities are based on the superposition of local models on the response system. The output layer only computes a linear combination of the activation of the neurons in the hidden layer. The activation of each neuron depends on the distance of the input vector to the prototype represented by the neuron. The radial activation function provides a nonlinear method of interpolating between the numbers of diverse fields (Brown and Harris, 1994). The technique always converges to the same point when trained with the orthogonal least squares algorithm. The advantage is that the network has decent global features with no local minima problem and good estimate value. This technique is widely used for modeling and classification application (Lin et al., 2013).

### 11.5.2 Validation and Error Estimation

The validation process will characterize the pattern recognition prediction model. The process evaluates the model's ability to perform recognition and classification of the unknown samples. The predictive ability of the instrument is evaluated based on the accuracy rate of correct classification of the unknown samples (Berrueta et al., 2007). This will prove that the classification models are able to perform the classification task. The validation can be performed either using external or cross-validation. The external validation uses separate data sets for training and testing. The cross-validation tends to use part of the data for training, while using another part of the data for testing the model (Berrueta et al., 2007).

The LOO method is one of the most widely used cross-validation approaches. The LOO method will remove one sample at a time from the training data and considers the remaining $n - 1$ samples as a test data (Basheer and Hajmeer, 2000). The validation method process will predict the classification performance based on the test data. The holdout method is based on random sampling without replacement. This method is a simple cross-validation technique used for model evaluation. In this method, the instances for the training set are selected randomly from the main data set and the remaining instances are selected in the testing data set (Kuncheva, 2004). The *k*-fold method is based on training data subsets drawn without replacement. This method splits the entire data set into *k*-blocks and each classifier is trained only on $k - 1$ blocks, while the remaining block is used for testing (Polikar, 2006).

## 11.6 RICE ANALYSIS: COMPARISON OF PERFORMANCE USING THREE DIFFERENT E-NOSES

Several settings that should be considered before the experiment is performed include the rice sample, the e-nose specific parameter settings, and the environmental conditions.

### 11.6.1 Sample Selection and Preparation

For this experiment, 17 different types of rice were selected. Of these, the samples were grouped into four categories known as ordinary rice (Calrose, Jati, and Jasmine), aromatic or milled rice (Maswangi, MRQ76, MRQ88, Jasmine, and Australia), brown rice (Fiona, Jasmine, Maswangi, MRQ76, MRQ88), and others (Moghul Basmathi, Herba Faiza, Thai Red, and local glutinous). Milled and brown rice of Maswangi, MRQ76, and MRQ88 were originated and cultivated at the Malaysia Agriculture Research and Development Institution (MARDI) Station. All the rice was stored in air-tight stainless steel containers to ensure no bias toward the storage effect.

The experiments were implemented in a closed laboratory. The laboratory temperature (22°C) and humidity (70%) were measured using hydrometer 506-HI from Testo UK. The controlled environment will minimize humidity and temperature variation during the experiment to ensure data repeatability.

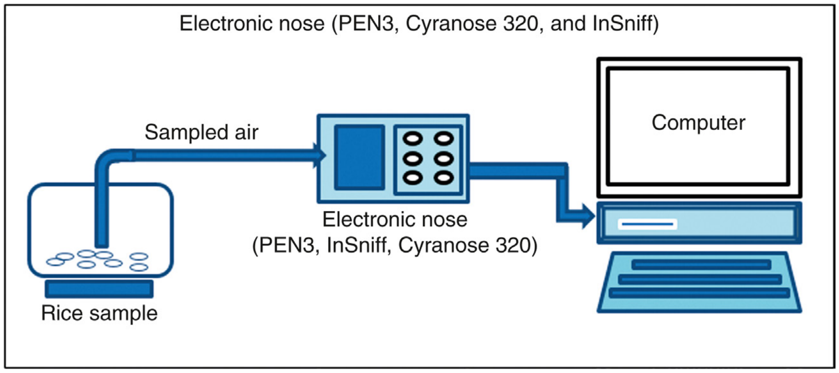
Electronic nose (PEN3, Cyranose 320, and InSniff)
Sampled air
Computer
Electronic nose
(PEN3, InSniff, Cyranose 320)
Rice sample

**FIGURE 11.1** Electronic noses setup for volatile compound evaluation of different types of rice.

About 50 g of each rice sample was weighed using an electronic balance and poured into a tin canister as shown in Fig. 11.1. The tin canister was wrapped tightly with a paraffin film wrapper. The sample was left idle for 10 min at room temperature for the sample to reach the equilibrium state before the experiment was performed.

## 11.6.2 The Electronic Noses

The experiments were performed using three different portable electronic noses: PNE3, Cyranose 320, and a custom-made InSniff. The instrument's main components include a sampling chamber, a sensing unit, an embedded control algorithm, and pattern recognition. The instruments are connected to a computer through serial or wireless communication. Each uses common sampling tools with unique operating procedure. Once data acquisition is completed, the acquired data will be used to analyze the odor samples.

### *11.6.2.1 Cyranose 320*

Cyranose 320 is a portable system from Smith Detection™ (Pasadena, CA, USA), consisting of 32 individual polymer sensors mixed with carbon-black composite. The polymer sensors are potentiometric sensors configured as an array. They are made up of various conducting polymers to sense a variety of volatile compounds. When the polymer sensors are exposed to an odor sample, each of its sensor array absorbs its specialized volatile compounds and swells like a sponge. During swelling, the distance between the conductive carbon-black particles increases and hence, increasing the resistance of the composite, which is measured as the sensor response. The PC Nose software is used to set up the instrument parameters, record, and analyze the acquired data.

### *11.6.2.2 PEN3*

The PEN3 is a portable electronic nose from Win Muster Airsense (WMA) Analytics Inc., Germany. The instrument comprises of a sampling tool, a chamber consisting of an array of sensors, and pattern recognition software (Win Muster v.1.6.2.14) for data acquisition. The sensor arrays are made up of 10 different metal oxide sensors (MOS). The sensor responses to specific volatile compounds indicated by the change of metal coating material conductivity. The measurement procedure and acquired data of the sensor response are controlled by Win Muster v.1.6.2.14 software.

### *11.6.2.3 InSniff*

Intelligent Sniffer (InSniff) is a portable electronic nose developed by UniMAP (Universiti Malaysia Perlis), Malaysia. It consists of 12 different MOS used to detect specific gases or volatile compounds. This sensor would respond to odor samples in volatile forms and convert it to electrical signals. The signals based on the electrical resistance of the sensor are proportionate to the samples' concentration. The sensors' response signals are measured by the biasing circuit voltage divider and are known as chemical "*fingerprints*." The measurement procedure and the acquired data of the sensor response are controlled by a custom-developed graphic user interface (GUI) program. The GUI program was developed using Visual Basic software version 6.0 from Microsoft (VB6.0).

## 11.6.3 Electronic Noses' Parameter Setting

The instruments applied static headspace sampling technique. The headspace gas was pumped into the sensor chamber at a constant rate via a Teflon-tubing connected to the instrument front-end during the data acquisition process.

Prior to the sample measurement, all electronic noses were subjected to preconditioning. Both PEN3 and InSniff electronic noses were switched on and conditioned for at least 30 min. For Cyranose 320, the sensor chamber was set to equilibrate at 30°C. The settings for the sampling process were set as in Table 11.2. In this work, preliminary experiments were performed to determine the optimal setup for the data acquisition process.

**TABLE 11.2** Parameter Settings for All the Applied Electronic Noses

| | | Cyranose 320 | | PEN3 | | InSniff | |
|---|---|---|---|---|---|---|---|
| | Cycle | Time (s) | Pump Speed (mL/min) | Time (s) | Pump Speed (mL/min) | Time (s) | Pump Speed (mL/min) |
| Sampling setting | Baseline | 60 | 120 | 30 | 400 | 30 | 400 |
| | Sample draw | 60 | 120 | 60 | 400 | 10 | 400 |
| | Idle time | 3 | — | — | — | 80 | — |
| | Purge | 120 | 120 | 120 | 400 | 60 | 400 |

The data acquisition process consists of a manual sequence of operations that includes purging and measuring. Before the sampling process, the instrument was purged using ambient air (filtered through activated charcoal) to ensure the instruments' sensor values are at baseline. During the measurement process, the headspace odor samples were pumped into the instruments' sensing chamber at a constant rate via Teflon tubing connected to the instrument's nozzle. Then the odor or volatile compounds in the sensing chamber were exposed and interacted with the sensor array. Once the sensor responses were stable, data were measured and recorded.

After the measurement process of each sampling, the instrument was again purged using ambient air (filtered through activated charcoal) to clean the sensor array for a period of time. This will allow the sensor array to recover to a stable state (the baseline value). The air filter used was made of activated carbon granules and had a large surface area, making it effective in removing a wide range of volatile organic compounds and moisture in the ambient air.

The instruments' baseline was measured prior to sample measurements and stored for later analysis. Each sample measurement was conducted in two different batches of samples and data acquisition measurements were replicated at least 10 times. In total, Cyranose 320 collected 4765 data measurements, while PEN 3 collected 6124 and InSniff collected 5955. During the data acquisition process, real-time data will be logged into a computer and later processed using MATLAB 2010 version 7 software.

## 11.7 RESULTS AND DISCUSSIONS

In this section, we discuss the experimental results using different classifiers such as MLP, SVM, *k*-NN, and RBF to classify 17 samples of rice according to 4 categories. The classification accuracy of each classifier is validated and compared, using three different error estimation approaches including LOO, holdout, and *k*-fold approaches. For the holdout approach, we partitioned the validation set using 60% of the available sample, while for the *k*-fold approach, we let $k = 10$ folds. In order to evaluate the validated classification accuracy of different classifiers for different error estimation approaches, the analyses for holdout and *k*-fold were executed five times and the overall results were presented within a certain range. The LOO approach was executed only once because the analysis was quite time demanding.

### 11.7.1 Cyranose 320 Data Analysis

Fig. 11.2 shows the correct classification rate of four different classifiers with respect to different error estimation approaches for four types of rice. It appears that SVM is the most consistent classifier across different error estimation techniques. The results of 100% correct classification for each error estimation method clearly proved SVM to be the best classifier. The second best classifier is *k*-NN based on the correct classification rates, followed by MLP and RBF. From the perspective of the error estimation approaches, it seems that LOO gave the most stable results across different classifiers. The results obtained by the LOO method confirmed this finding. For instance, this method provides the least error for MLP (0.02%), SVM (0%), *k*-NN (0%), and RBF (0.69%). Even though this method was executed only once, the average error rate scores for holdout and *k*-fold methods were still higher than that of the LOO method. The next best error estimation is presumably *k*-fold followed by the holdout method.

### 11.7.2 PEN3 Data Analysis

Findings for PEN3 were almost the same with those for Cyranose and InSniff. SVM consistently appeared to be the most powerful classifier for all three sensors and for all the error estimation approaches. Error rate for LOO remained perfect, however, the error rates for holdout and *k*-fold for MLP, *k*-NN, and RBF were recorded higher than those for Cyranose 320 and InSniff. Again, across all types of error estimation, *k*-NN is considered as the second best classifier, followed by RBF and MLP. The identification rate degraded a lot when holdout and *k*-fold were applied to validate the classification accuracy. From the viewpoint of

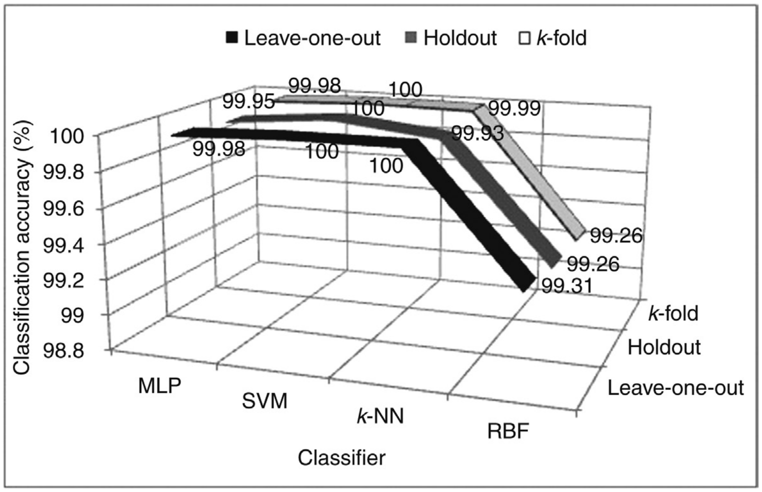

**FIGURE 11.2** **Validated correct classification rate of MLP, SVM, *k*-NN, and RBF using LOO, holdout, and *k*-fold error estimation for Cyranose 320 data.**

error estimation approaches, the LOO method proved to be better than the others. The *k*-fold is still considered reliable for error estimation since at most only 0.23% error was committed across all the classifiers, whereas the holdout approach gave 2.28% error specifically for the MLP classifier. These results are illustrated in Fig. 11.3.

Generally, the best classifier for all the three sensors data appears to be SVM, followed by *k*-NN, RBF, and MLP. However, for Cyranose 320, MLP is better than RBF. The best error estimator turned out to be the LOO method with mostly perfect identification, except for Cyranose using MLP and RBF classifiers as well as the PEN3 for the MLP classifier. The second best classifier generally is *k*-fold, followed by the holdout method based on the error rate score across all the classifiers.

## 11.7.3 InSniff Data Analysis

Fig. 11.4 illustrates the identification rates of four types of rice using InSniff. From the perception of classifier, it seems that again SVM recorded the highest correct classification rates with no error for each error estimation method. Then, *k*-NN emerges as the next best classifier with the error rate of 0.01% for the holdout and the *k*-fold methods, respectively. This is followed by MLP and RBF for error estimation using the holdout method with the difference of 0.03%. However, if *k*-fold was applied, RBF turned out to be better than MLP with a 0.2% difference in error. Generally, the best error estimation that suits the classification of InSniff data is the LOO approach with no error, followed by holdout and *k*-fold approaches with an error rate at most 0.05 and 0.25%, respectively.

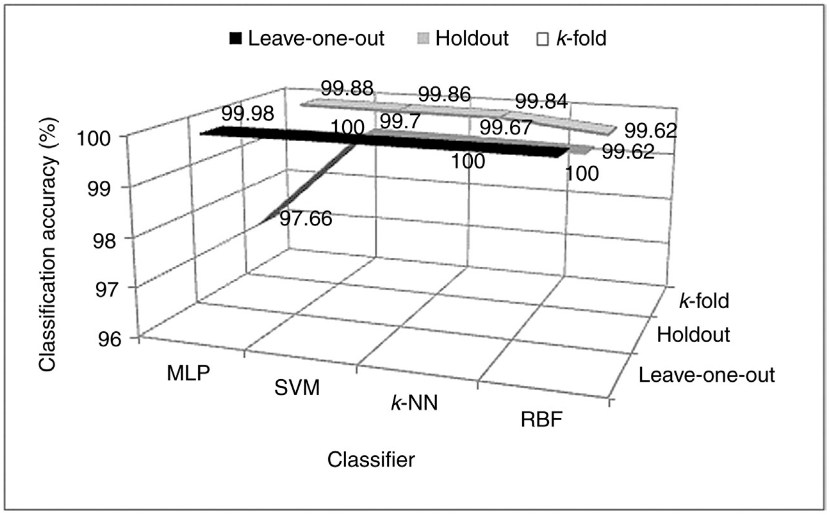

**FIGURE 11.3** **Validated correct classification rate of MLP, SVM, *k*-NN, and RBF using LOO, holdout, and *k*-fold error estimation for PEN3 data.**

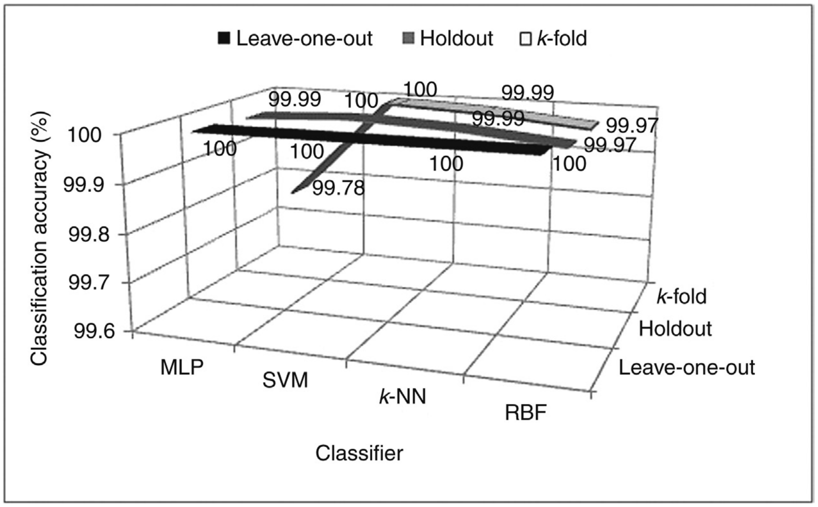

**FIGURE 11.4** **Validated correct classification rate of MLP, SVM, *k*-NN, and RBF using LOO, holdout, and *k*-fold error estimation for InSniff data.**

## 11.8 CONCLUSIONS

The objective of this study is to classify 4 groups of 17 types of rice using 3 different portable electronic noses. The discrimination of these rice types was performed using four different classification approaches—MLP, SVM, *k*-NN, and RBF. In these approaches, the aim was to find the best classifier~~s~~ that can produce the least classification error. Three different error estimation techniques were applied for each classifier—namely, LOO, holdout, and *k*-fold. The findings of the analyses show that generally the best classification result can be obtained using SVM with the LOO error estimation approach. However, *k*-NN, RBF, and MLP also yielded reasonable results. It suggests that LOO is the best error estimation technique for rice classification. These findings imply the successful application of ordinary portable electronic noses in classifying different types of rice into their correct groups. It also proves that all the applied electronic noses are able to identify different types of rice with good classification performances. Furthermore, the application of the electronic noses can also be used for rice plant disease and rice mold detection. The application of these instruments may offer good potential in enhancing the production and may also increase rice yield.

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## Chapter 12

# Electronic Noses for the Quality Control of Spices

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## 12.1 INTRODUCTION TO SPICES AND CULINARY HERBS

Spices are parts of plants, for example, buds and flowers, seeds and fruits, herbs and leafs, roots and rhizomes, bulbs and barks, which are added to foodstuffs due to their natural flavoring, their aromatizing, and coloring properties. Spices can improve digestibility of food and extend the shelf life of food because of their antimicrobial or antioxidant properties. There exist a great variety of herbs and spices, in use for thousands of years (Peter, 2001). The European Spice Association (ESA) listed over 45 important herbs and spices (ESA, 2014). More than 2 million tons of spices, most of them from India, were worldwide produces per annum. The most important spices include chillies and pepper, cumin, ginger, cinnamon, and nutmeg.

The flavor of spices is a sensory impression and caused by many volatile organic compounds, which can be concentrated by solvent extraction or water steam distillation in hydrophobic liquids, so-called essential or ethereal oils. Most of the aroma compounds are terpenes (eg, pinene[a]), sesquiterpenes (eg, caryophyllene), terpenoids, or unsaturated cyclic (phenolic) compounds. Artificial aroma compounds, also called natural-identical, that is, chemically defined substances with aroma properties, prepared by chemical syntheses or biotechnical methods are not considered in this chapter.

The properties of spices, such as flavor, color, and pungency varies among cultivars, species, and harvests. The aroma compounds may be subject to chemical reactions, resulting in changes of its flavor during ripeness, harvest, treatments (eg, drying, fermentation), and transportation. The properties of spices change during storage influenced by temperature, humidity, and light. Evaporation of flavorings, autooxidation, enzymatic browning, and microbiological processes are responsible for changes in properties. Many spices are highly sensitive to fluctuating pH levels, and rapidly break down chemically in the presence of light and oxidizing agents. Therefore, a quality control of spices is indispensable in the logistic chain from the producer, to the trader, to the supplier, to the seller. Minimum quality requirements are stipulated in a document by the ESA and the International Organisation of Spice Trade Association (IOSTA, 2008). Substantial contributions to the quality assessment of spices can be delivered by electronic noses because they are not very cost-intensive devices. In most cases, electronic noses were deployed together with other methods of sensory analysis (olfactometry) as well as of chemical and biological analysis. These well-established methods, which are needed as a reference and for calibration of electronic noses, are briefly reviewed in this chapter.

## 12.2 CLASSICAL METHODS OF SPICE ANALYSIS

The odor and taste of spices is analyzed and assessed by a panel of trained persons in a defined smelling and tasting test (ISO, 2007, 2012). The descriptive analysis is used to assess spice characteristics; floral, herbal, spicy, or earthy. Also the intensity of the flavor is tested by quantitative descriptive analysis, for example, in a scale from 0 to 6. These methods are described in several publications and normalized in standards (Derndorfer and Baierl, 2006; DIN, 1997, 2014). However, critical investigation of sensory analysis indicates there are large uncertainties about these methods, which restrict their potential use as reference for machined olfaction (Boeker, 2007).

The chemical compounds that primarily establish the characteristic flavor are found in the essential oil of the spices. The quantity of essential oil is commonly a measure of the spice quality. A classical steam distillation procedure for the determination of volatile oil in spices is used

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[a] Many of these compounds exist as isomers. For details of this structural differences and their impact on flavor, see the cited references.

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(ISO, 2008). The extracted essential oil can be analyzed by high-performance liquid chromatography equipped with a UV detector (HPLC–UV) (Berger, 2007; Ötles, 2008). The analysis of flavor-giving volatile organic compounds in the headspace of a spice sample can be performed by gas chromatography using a flame ionization detector (GC–FID) or mass spectrometer (GC–MS) (IOFI 2012). These methods were also used for detection of unwanted contaminations such as pesticides.

Gas chromatographic–olfactometry (GC–olfactometry) combines the separation of flavoring compounds from a mixture and their identification according to the retention time with an olfactoric description (Blum, 1999). Thoroughly trained personnel sniff the volatiles emerging at different retention times from an exit port of the GC and deliver appropriate smell descriptions.

Further quality parameters such as the content of ash, moisture, water activity, and bulk density have to be determined according to agreed methods. The water content has to be determined because the moisture content in spices has an influence on odor and taste as well as on stability against microbiological infestation.

Spices as natural products are in contact with their environment and can be contaminated with toxin-producing microorganisms. To evaluate the microbiological infestation, the colony counting method is applied and the number of colony-forming units (CFU) per mass after incubation is determined.

All these methods are performed discontinuously in a laboratory, requiring equipment and qualified personnel, and are time consuming.

## 12.3 DEPLOYMENT OF ELECTRONIC NOSES FOR SPICE ANALYSIS

An electronic nose—a device for machined olfaction—is essentially a multi-gas sensor. It can only differentiate, identify, or quantify gases or gas mixtures. It becomes an electronic nose only if it can provide information about the odor of a gas mixture. A correlation to odor can be built by using reference data derived from the human sense of smell.

For the evaluation of the results from spice analysis by electronic noses, it should be considered that the smell and taste senses of humans are based on a multitude of chemoreceptors, developed and adapted for specific functions over thousands of years. Smell analogous measurement and the automated simulation of the sense of smell are complex. Additionally, it is challenging to analyze a large number of compounds with varying concentrations which constitute a single flavor.

The user of an electronic nose should be aware that the deployment of an electronic nose for an odor analysis demands in many cases, an expensive and tedious preinvestigation to prove the applicability along with a systematic method development and validation.

One of the first investigations on spices was published in 2000 (Nitz, 2000), highlighting on the basis of selected examples, the possibilities and limitations of application of a sensor system for detection and/or quantitative evaluation of flavors, to quantitatively elucidate aroma compositions, flavors, and off-flavors. Until now, about 40 investigations on spices and herbs using electronic nose were published according to a search in "web of science." Table 12.1 is a compilation of applications by electronic noses for spice analysis. Even if flavor description was not provided, the investigations contributed to quality control of spices.

## 12.4 CASE STUDIES FOR THE ANALYSIS OF SPICES

### 12.4.1 Identification of Different Types of Spices

The capability to differentiate between different types of spices was demonstrated in a study by Zhang et al. (2003). An electronic nose based on 12 conducting polymers (CP) was used to distinguish among 4 types of spices (basil, cardamom, pepper, and turmeric were purchased from the local store). The discrimination analysis with neuronal networks with multilayer perception (ANN) was applied for data analysis. The results showed that the applied mathematical methods delivered in 4 min a correct recognition of the spices from 60–100%.

A further investigation was performed to evaluate several of Thai herbs (Ayudhaya et al., 2009). The essential oils volatilized from selected fresh herbs were compared using a low-cost electronic nose containing 10 metal oxide semiconducting sensors (MOX). The signal response of the electronic nose was evaluated by principal component analysis (PCA). PCA clearly distinguished all the samples. In cross-validation, more than 97% of the groups were correctly classified.

Discrimination among six complex odors from saffron, spearmint, cumin seed, cinnamon, golpar (*Heracleum persicum*), and thyme was performed using a single generic tin oxide gas sensor (Hossein-Babaei and Amini, 2014). The sensor underwent four step-like temperature jumps in a test period of 4 s. Linear discriminant analysis (LDA) was used for mapping the preprocessed sensor data in a three-dimensional feature space. Tests over 6 months show that the odor discrimination is aging drift-proof.

### 12.4.2 Red Pepper

Red pepper is a term for spices of the genus *Capsicum* L. It describes the fruits of flowering plants of the nightshade family Solanaceae, native in South America. The fruit of *Capsicum* plants occurs in several varieties with different

**TABLE 12.1** Overview on Deployment of Electronic Noses for Spice Analysis

| | Deployment | | Electronic Nose | | |
|---|---|---|---|---|---|
| | **Task** | **Case Study** | **Sensing Elements** | **Data Evaluation** | **Sources** |
| 1 | Identification of different types of spice | Basil, cardamom, pepper, and turmeric | 12 CP | ANN | Zhang et al. (2003) |
| | | Thai herbs | 10 MOX | PCA | Ayudhaya et al. (2009) |
| | | Saffron, spearmint, cumin seed, cinnamon, golpar (Persian hogweed), thyme | 1 MOX, thermal cycling | LDA | Hossein-Babaei and Amini (2014) |
| | | Ternary model spice mixtures | 12 CP | ANN | Zhang et al. (2005a,b) |
| 2 | Identification of the origin | 4 pepper samples of different regions of India | 12 MOX | PCA | Mamatha and Prakash (2011) |
| | | 13 saffron from different countries | 27 MOX | PCA | Carmona et al. (2006) |
| | | 8 saffron samples of different brand and country | 5 MOX<br>10 MOX | PA, SVM<br>PCA | Tahri et al. (2015)<br>This paper |
| | | 8 cumin samples of different countries | 5 MOX | PA, PCA, SVM | Ravi et al. (2013) |
| | | 8 samples of essential oil from coriander | 12 MOX | PCA | Ravi et al. (2007) |
| | | 8 garlic samples | 8 QMB eight + 8 MOX | LDA | Baby et al. (2009) |
| 3 | Differentiate between different quality of spice samples | 8 samples of cumin from India | 12 MOX | PCA | Ravi et al. (2013) |
| | | Oregano essential oil from different plant species | MOSFET + MOX | PCA | Seregely and Novak 2005 |
| | | 3 cardamom samples from different regions of India | | PCA | Ghosh et al. (2012) |
| | | Garlic | 6 MOX | PA | Tamaki et al. (2008) |
| | | *E. splendens* flavored oils | 6 MOX | PCA | Chung and Lee (2002) |
| | | *Cnidium officiale* | | PCA | Lee and Chung (2002) |
| | | *Eurycoma longifolia* extracts | QMB | PCA | Islam et al. (2006) |
| | | *P. frutescens* L. discriminate three different cultivars | 10 MOSFET + 12 MOX | PCA | Laureati et al. (2010) |
| 4 | Differentiate purgency | Ground red pepper | 12 CP | DFA | Korel et al. (2002) |
| | | Powdered red pepper | MS | DFA | Soo et al. (2010) |
| | | Black pepper powder and essential oil | 12 MOX | PCA | Mamatha and Prakash (2011) |
| 5 | Identification of adulterations | Spice mixtures for saveloy and sausages and garlic powder | 30 MOX<br>IMS | LDA<br>PCA | Banach et al. (2009) |
| | | Black pepper | IMS | PCA | Tiebe et al. (2014) |
| 6 | Identification of contaminations | Mold's growth on sweet pepper | 38 MOX | LDA | This paper |
| | | Mold's growth on nutmeg | 38 MOX<br>IMS | LDA<br>PCA | This paper |
| 7 | Indication of treatments | Milling of black, white, and green pepper | 6 MOX | PCA | Liu et al. (2013) |
| | | Gamma-irradiation of Korean red pepper powder | 12 MOX | PCA | Lee et al. (2004) |
| | | Electron beam radiation of two cumin powders and red chilli powder | SAW | PCA | Sanyal et al. (2014) |
| | | Irradiated garlic | SAW | PCA | Kim et al. (2014) |

composition of the aroma-giving ingredients. It has a variety of names depending on the origin and type, such as red pepper, cayenne pepper, chilli and pepperoni. Slightly spicy species are bell or sweet pepper and paprika.

The alkaloid capsaicin and minor capsaicinoid substances create the burning sensation by irritation when it comes in contact with mucous membranes. The content of these components in the fruits ranges from 0.01% (bell pepper) to 10% (hot chilli).

An electronic nose based on 12 conductive polymers was used to discriminate ground red pepper samples obtained from local retail markets in Izmir, Turkey, by headspace volatiles (Korel et al., 2002). Samples of the different ground red pepper were characterized with high HPLC to determine the capsaicin, dihydrocapsaicin, and total capsaicinoid amounts. Scoville scores, a measure of pungency, was determined by means of sensory tests (ISO, 1995). A linear correlation between the amount of capsaicinoids and the Scoville scores was observed. Electronic nose data obtained from the different red pepper samples were analyzed using discriminant function analysis (DFA) as a pattern recognition technique. An overall correct classification rate of pepper varieties by electronic nose of 91% was obtained.

An electronic nose was used for an assessment of grading pungency on powdered red pepper (Soo et al., 2010). Mild and powdered pungent red peppers were mixed at concentrations of 0, 25, 50, 75, and 100%. The mixtures were analyzed using a mass spectrometer-based electronic nose. Discriminant function analysis (DFA) was conducted on electronic nose data. The discriminant function first (DF1) score values decreased with increasing amount of powdered red pepper with a pungent taste. Discriminant function second (DF2) score values moved from the negative position into the positive position with increasing the amount of red pepper powder. The results obtained by an MS-based electronic nose agreed with that of the HPLC, indicating an increase in concentration of capsaicin.

*Capsicum* can be infested by mold fungi that produce mycotoxins. The mold growth starts after an incubation time of one to two days, increases, reaches a maximum, and decreases due to a lack of consumable matter. This cyclic mold growth can be detected from the presence of gaseous metabolites, so-called microbial volatile organic compounds (MVOC) (Tiebe et al., 2009). The molds produce various volatile compounds of different type and quantity, depending on their variety and growth cycles. In order to investigate this phenomena, a powder of sweet red pepper (*Capsicum annuum*) was inoculated with the mold species *Aspergillus flavus*. Gas samples were taken from the headspace of the infested spices and analyzed by an electronic nose with a chip array of 38 sensing elements, based on gas sensitive semiconductive tin oxide (KAMINA-type). The data evaluation of the measured the changes in resistance of the sensing elements of the electronic nose by LDA permitting a differentiation between samples of different stages of mold growth of *A. flavus* on sweet pepper. The results showed that the electronic nose can be used for detection of mold in spice samples and that the mold's growth can be indicated after an incubation time in a very early state before it becomes visible. In addition, a headspace analysis was performed by GC–MS and the characteristic volatile metabolites from the mold's growth, such as butanol, 2-methylpropanol, methylfuran, and acetic acid, were identified.

### 12.4.3 Black Pepper

The fruits of pepper (*Piper nigrum* L.), a shrub of the Piperaceae family, is an extensively used spice. Green pepper is the unripe and fast-dried fruit; black pepper is also unripe fruit, which developed a black skin by cooking and drying. White pepper is the ripe fruit without colored skin; orange and red peppers are specifically prepared from ripe fruit.

Essential for the pungency of pepper are piperine, and the piperine derivatives piperettine and piperyline. The amount of essential oil, which also gives the flavor, is about 2.5% in white pepper, and in green pepper and black pepper up to 4.8%. The essential oils contain terpene such as caryophyllene, caren and limonene, pinene, sabinene, and linanool. Further components are rotundone, a sesquiterpene, which contributes to the pepper aroma.

Four pepper samples from different regions and their extracted essential oils were investigated using an electronic nose, a sensory analysis for flavor and odor profile, as well as GC–MS (Mamatha et al., 2008; Mamatha and Prakash, 2011). The flavor profile of pepper powder and of the essential oils clearly differentiates one sample from the three other samples. The electronic nose pattern confirmed the results from sensory and GC–MS data. The intensity of pepper pungency was estimated by sensory and instrumental analysis, and by using an electronic nose. The sample with lower spicy and pungency odor attributes and a lower Scoville index was clearly identified by electronic nose analysis.

Adulterations of pure pepper powder can occur on the market. An investigation was performed using ion mobility spectrometry (IMS) and an electronic nose in order to identify falsification. Black Brazilian pepper and a sample blend by addition of 10% of mustard flour were investigated (Tiebe et al., 2014). The sensor responses of the electronic nose were used for linear discriminant analysis. Fig. 12.1 shows the LDA scores of four pepper samples—black Brazilian pepper as the reference sample, adulterated black Brazilian pepper (10% mustard flour), and black and white Indonesian pepper. The first two discriminant axes capture 84.4% of the variance. The LDA indicates a clear separation of the four pepper samples. There is discrimination between the Brazilian pepper sample and the adulterated sample as well as the samples of white and black Indonesian pepper.

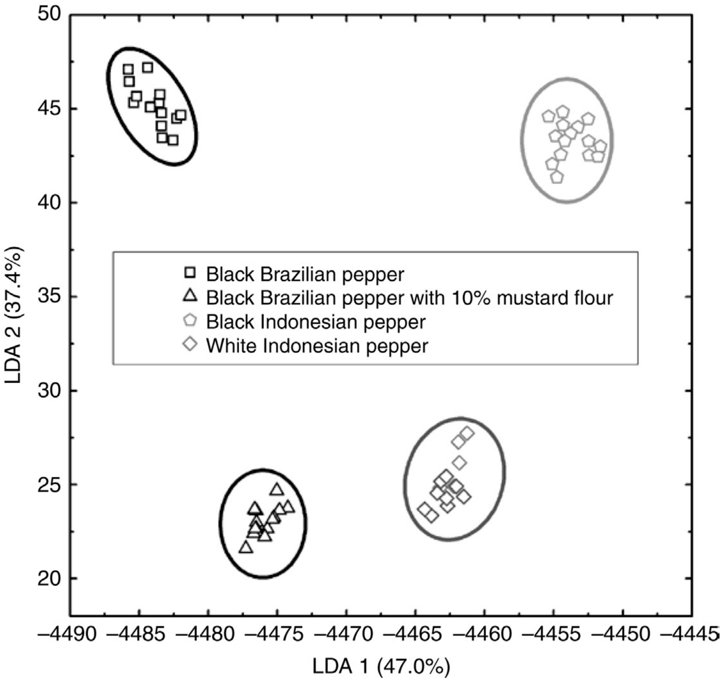

**FIGURE 12.1** LDA results of electronic nose response on pure Brazilian and Indonesian pepper and adulteration with 10% mustard flour.

## 12.4.4 Nutmeg

The seed of the tree *Myristica fragrans* delivers the nutmeg surrounded by seed coat, called mace. The content of essential oil of nutmeg is in the range of 5–13%. The characteristic aroma results from the terpenes, such as pinene, sabinene, limonene, borneol, terpineol, eugenol and isoeugenol, camphene, and phellandrene.

Unfortunately, nutmeg is, in many cases, infested by molds that produce carcinogenic aflatoxins. In order to study mold infestation, nutmeg powder was infested with *Penicillium verruculosum.* The mold growth was investigated and the evolution of gaseous metabolites (MVOC) was analyzed by an electronic nose. The data evaluation of the measured changes in resistance of an electronic nose containing 38 semiconductive sensing elements by LDA permits a differentiation between the samples of different stages of mold growth. Fig. 12.2 shows the LDA plot of headspace above nutmeg infested in a period of 9 days.

The results of the electronic nose investigations show that MVOC can be detected in an early state of mold growth even when mycelium growth was not visible.

## 12.4.5 Saffron

Saffron (*Crocus sativus* L.) is a flower from the Iridaceae family, native from Greece to Southwest Asia. Each flower has three crimson stigmas, which are used as a spice and a coloring agent. Saffron is among the world's most costly spices by weight because 1 kg requires about 110,000–170,000 flowers. The orange or red color results primarily from crocin. More than 150 compounds were detected, significant among which were the aroma-yielding compounds; safranal and picrocrocin.

An analysis of the volatile fractions of saffron from different origin was performed with an electronic nose and GC–MS (Carmona et al., 2006). The electronic nose contains 27 commercial available gas-sensing elements based on metal oxides. Sensor responses were displayed in polar plot and data analysis was done using PCA that takes into account the first 10 coefficients of the fast Fourier transform of the curves as the input variable. The electronic nose was capable to differentiate between the countries where saffron samples originated with a confidence of 90%. This coincides with the results from GC–MS, which is capable of differentiating saffron by its origin.

In a further study on saffron samples from different origins and harvest, an electronic nose containing 10 semiconductive metal oxide sensors was used. The data obtained were analyzed using LDA analysis and plotted in Fig. 12.3. The results clearly distinguished the saffron samples from Syria and Iran from those of Morocco (Tallouine). Comparable results were recently obtained by Tahri et al. (2015), using a voltammetric tongue and an electronic nose based on a five-sensor array (MOX).

Hence, these studies gives further evidence that the electronic nose could be useful for the discrimination of saffron origins and could provide an alternative to the traditional analytical methods.

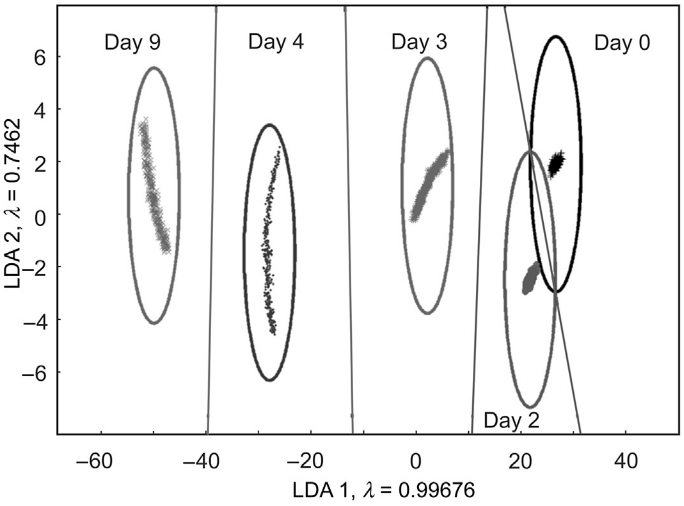

**FIGURE 12.2** LDA plot of electronic nose data from head-space analysis of nutmeg infested with *P. verruculosum* during 9 days.

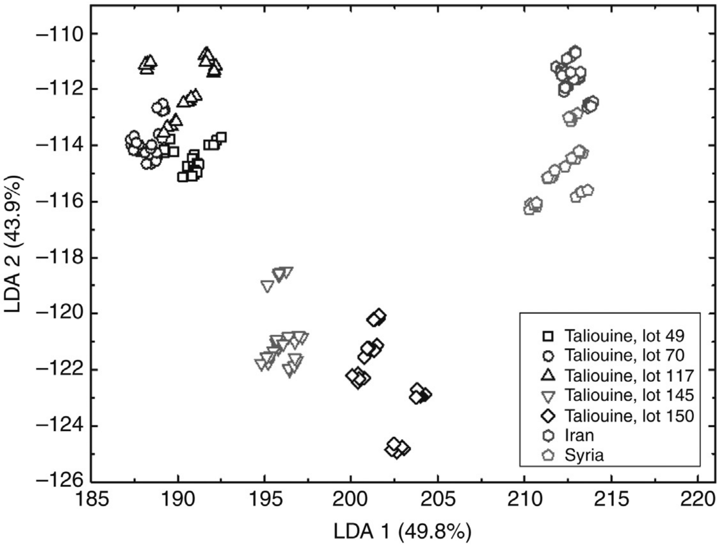

**FIGURE 12.3** LDA plot of electronic nose investigations from seven saffron samples.

## 12.4.6 Cumin

The flowering plant (*Cumin cyminum* L.) from the family of Apiaceae. Ground cumin is used as a flavoring agent in a number of ethnic cuisines. The chemical entities which primarily establish its characteristic pungent flavor are found in the essential oil of cumin. The flavor comes in particular from cuminaldehyde, cuminic alcohol, pyrazines, terpinene, safranal, cymene, and pinene.

An investigation of eight samples of cumin essential oils grown in different regions of India was carried out by GC–MS, GC–olfactometry, sensory profiling, and electronic nose techniques (Ravi et al., 2013). The main aroma components were identified by GC–MS headspace analysis. Sensory odor profiling indicated that two samples had significantly higher intensity of floral, cumin-like, and citrus aroma notes. These two samples that slightly differ in chemical composition were also separated from the other samples by electronic nose investigations and PCA signal analysis.

## 12.4.7 Cardamom

The fruits of cardamom (*Elettaria cardamonum* L.), a plant of the Zingiberaceae family, are used as a flavoring agent and drug in traditional medicine. Today, Guatemala is the largest producer of cardamom, which is one of the most highly priced spices in the world. The essential oil of the fruit contains above all terpenylacetat, terpineol, mycrene, limonene, linalylacetat, linalool, sabinene, cineol, and hydroxycinnamon acid.

An aroma quality estimation was performed on three clone-specific cardamom samples by electronic nose (Ghosh et al., 2012). The PCA showed three distinct clusters in the plot of the first two principal components PC1 91.6% and PC2 6.8%. The study demonstrated the rapid differentiation of samples and quality estimation of cardamom by electronic nose.

### 12.4.8 Coriander

Coriander (*Coriandrum sativum* L.) is a plant from the family of Apiaceae. Seeds and the herb of coriander, both of which are used as spice or a medicinal plant. It contains flavoring compounds such as linalool, geraniol, pinen, limonene, geranylacetat, terpinen, and borneol.

An electronic nose with metal oxide semiconducting sensing elements was deployed for the investigation of coriander essential oil samples from eight regions of India (Ravi et al., 2007). The results of headspace investigation were analyzed by PCA showing the discrimination in odor profile of coriander samples of different regions. The PCA mapping clearly distinguished the samples in relation to their dominant volatile compounds determined by GC–MS headspace analysis. This is in agreement with results from quantitative descriptive sensory analysis and from GC–olfactometry investigations.

### 12.4.9 Oregano

Oregano (*Origanum vulgare* L.) is a spice and medical plant from the Lamiaceae family. Over 60 different compounds have been identified with the primary ones being thymol and carvacrol. Further components are *p*-cymene, caryophyllene, spathulenol, germacrene-D, fenchyl alcohol and terpineol, terpinene, pinene, and limonene.

The herbs of four *Origanum* species (three *Origanum vulgare* subspecies *hirtum* and *O. vulgare*) that had grown in the same season were examined by GC and electronic nose (Horvath et al., 2002). The subspecies *hirtum* clones contained a higher amount of essential oil (3–4%) in comparison to *O. vulgare* (0.2%) in full flower. Regarding the essential oil components, all samples had the same quantity of carvacrol, while one selected subspecies *hirtum* line showed significantly more cymene and terpinene. The complex of aromatics was different for all selected lines according to distinct sensor signals of the electronic nose.

A parallel quality investigation of various oregano species was performed by sensory analysis, gas chromatography, and electronic nose using PCA for data evaluation (Novak et al., 2003). The GC analysis of essential oil identified main components and revealed differences between plant species (*O. vulgare* subsp. *hirtum* and *O. majorana*). The instrumental and human sensory analysis showed similar results and varieties of oregano species (*O. majorana*) could be well distinguished on the basis of their complex aroma, whereas their gas chromatograms did not show characteristic differences.

The essential oil of oregano samples and dried root samples of lovage (*Levisticum officinale*) harvested at different times and from the 2- and 3-year-old population were investigated as a comparative analysis with electronic nose which consisted of metal oxide silicon field effect transistor (MOSFET) sensors and metal oxide sensors (Seregely and Novak, 2005). The sensor responses were evaluated by PCA, canonical discriminant analysis (CDA). The best separation was achieved by combination of both methods. In all cases, more than 90% of cross-validated grouped cases were classified correctly.

### 12.4.10 Garlic

The onion from garlic (*Allium sativum* L.) and sometimes seeds as well as sprouts are used as spice and medication. The sulfur-containing allicin, which is the characteristic garlic compound, is formed from the odorless alliin by cracking the garlic cell structure.

The possibility to characterize the garlic odor in vitro (in head space) and in vivo (breath from a person who ingested garlic) using electronic nose was demonstrated by Tamaki et al. (2008). The electronic nose based on an array of metal oxide semiconductor sensors can differentiate between the various garlic-associated odors corresponding to the different origins, or to different processes, that is, raw or heat-treated.

The correlation between volatile compounds identified by gas chromatography and the responses of human perception in sensory analysis are discussed. Results showed that odor sensor data by electronic nose were easier to obtain and were well correlated with both GC and sensory analysis.

An electronic nose was used to study the odor profile of eight garlic samples from different origin (Baby et al., 2009). Samples were oven-dried or treated specifically (lyophilization in liquid nitrogen) to preserve flavor. The electronic nose consisted of two sensor system modules, one contained eight polymer-coated quartz microbalance (QMB) sensing elements and the other had eight semiconductive tin oxide sensing elements. Sensor signals were processed statistically by LDA. Pattern recognition and multivariate analysis of the electronic nose data were able to separate the garlic cultivars easily. Lyophilized, oven-dried, and humidified specimen samples also were clearly discriminated.

### 12.4.11 Exotic Herbs

Essential oils from seeds of black-caraway (*Nigella sativa* L.) were investigated by gas chromatography, olfactometry sensor profile analysis and an electronic nose with

18 MOX sensors using PCA for differentiation of 5 samples (Zawirska-Wojtasiak, 2010). Further investigations were performed on herbs and their essential oils, typically used in Asia for flavoring food or for traditional medicine, such as *Elsholtzia splendens* (Chung and Lee, 2002), *Cnidium officinale* (Lee and Chung, 2002; Islam et al., 2006), and *Perilla frutescens* L. (Laureati et al., 2010). Electronic noses based on metal oxide sensors or quartz crystal microbalances (QCM) were used. PCA was deployed for data analysis to differentiate between samples of different cultivars and shelf life.

### 12.4.12 Processing of Spices

Spices are processed after harvest by drying, fermentation, milling, and chemical treatment (eg, fumigation) and radiation for conservation, flavor development, conditioning, and packaging. All of these processes have an impact on the quality parameter of spices and need to be monitored.

The electronic nose was deployed in a study to compare the effects of cryogenic grinding and hammer milling on the flavor attributes of black, white, and green pepper (Liu et al., 2013). Pattern recognition based on electronic nose data supported sensory and instrumental findings. The flavor attributes analyzed by headspace solid-phase microextraction (HS–SPME) and GC–MS, and sensory evaluation showed that cryogenic grinding resulted in minimal damage to the color, flavor, and sensory attributes of the spices. Cryogenic grinding was also better than hammer milling in preserving the main potent aroma constituents. However, it was found that the flavor quality of ground pepper was decreased during storage.

Spices as a natural product can be exposed to microbial contamination during harvesting and storage. They may contain soil-borne bacteria, fungi, and insects because many of them are dried in the open air. Therefore, spices and herbs are currently treated with ionizing radiation to eliminate microbial contamination. Treatment with ionizing energy seems to be more effective against bacteria than thermal and chemical treatments, and it does not leave chemical residues in the food product.

The effect of gamma-irradiation on color, pungency, and volatiles of Korean red pepper powder (*C. annuum* L.) was investigated (Lee et al., 2004). The red pepper powder was irradiated by a gamma radiation dose up to 7 kGy. The effect of gamma-irradiation on color, pungency, and volatiles was investigated using various methods and an electronic nose with metal oxide sensors. An irradiation dose of 7 kGy of red pepper powder reduced the population of bacteria and fungi effectively without affecting major quality factors. The red color of irradiated pepper powder was not significantly different from that of the nonirradiated sample. Pungency of irradiated red pepper powder was not changed. Odor profiles were classified into irradiated dose levels of 0, 3, 5, and 7 kGy by PCA and multivariate analysis of variance was performed. A difference in odor might result from the disappearance of some volatiles. Moreover, it appears that the irradiation of packaging material induced new substance formation, which migrated into the red pepper powder.

For the investigation of the impact of electron beam irradiation on cumin and red pepper, an electronic nose based on surface acoustic wave (SAW) sensors, as well as FTIR and EPR spectroscopy, was applied (Sanyal et al., 2014). Two different cumin powders and red chilli powder all from India were irradiated with doses of 6, 10, and 14 kGy. Different fingerprints of the polar plot were obtained in the irradiated spice samples compared to the nonirradiated ones. The PCA technique was used to get a clearer trend of the numerical data. A clear distinction between nonirradiated and irradiated samples was observed for all the samples. The most promising results were found for red chilli powder.

An electronic nose was used for the identification of frozen crushed garlic samples produced in Korea and China (Kim et al., 2014). The samples were irradiated (electron-beam and gamma-rays) with 1, 4, and 7 kGy. It was shown that irradiation treatments unequivocally reduced the microbial populations with dose increments and a microbiological screening effectively differentiated the nonirradiated and irradiated samples. The electronic nose positively differentiated the odor patterns of samples based on PCA.

### 12.4.13 Spice Mixtures

The mixing of spices is an old tradition and classical mixtures such as curry or chilli powder and many local mixtures are in common use. The quality of spice mixtures may differ because of amount and quality of various components. Distinct changes in composition of spice mixtures, which were not easily detectable, can be indicated and documented by electronic nose investigations.

Ternary model spice mixtures were investigated by Zhang et al. (2005a,b). The samples were presented to an electronic nose with 12 conducting polymers. Data analysis was performed using neuronal networks with multilayer perception. Basil, cinnamon, and garlic were mixed in different compositions and analyzed an electronic nose. The results showed that mixtures of the three spices in different quantities could be identified. Both sensory and gas chromatography analysis were performed. The accuracy and efficiency of gas chromatography and sensory methods in predicting spice mixture compositions were investigated and compared with those of an electronic nose. Triangle tests were performed to estimate different thresholds for spice mixtures. It was found that the prediction errors and thresholds of the electronic nose methods were lower than those of sensory analysis. The GC method provided a more accurate but much less efficient prediction.

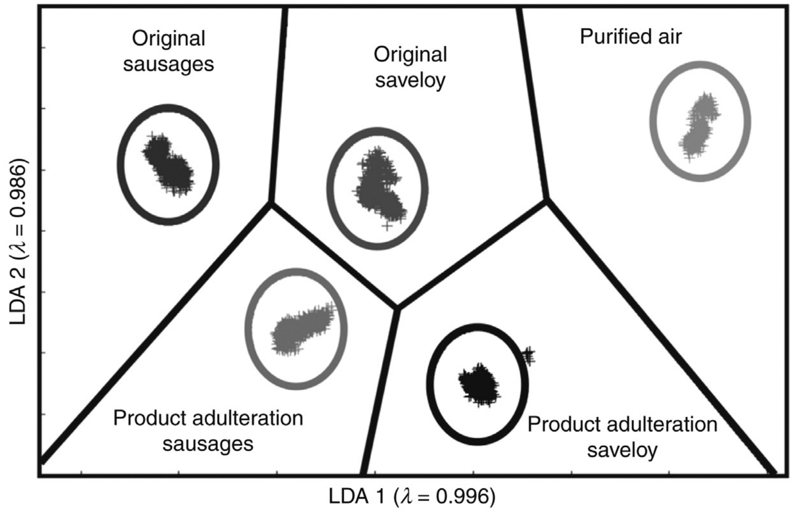

**FIGURE 12.4** **The results of LDA analysis of electronic nose data (KAMINA type) for distinguishing spice mixtures.**

Electronic noses can be deployed to determine differences between original and product adulterations of spice mixtures. This was demonstrated for two different spice mixtures delivered from Kahler Gewürze GmbH, Germany (Banach et al., 2009). The first sample was a "saveloy" spice mixture and the fraud contained an admixture of 20% of a curry spice. The second was a "sausages" spice mixture and the adulteration contained 80% original spices and 20% of garlic powder. Original and adulteration always had the same color. The volatile organic compounds in headspace of spice mixtures were detected by GC–MS to validate the differences in composition. The amount of these components differed in the original and the adulterated samples of spice mixtures. An electronic nose with a chip array of 38 sensing elements was applied. The results of an LDA analysis are shown in Fig. 12.4. The results obtained from a headspace detection of volatile organic compounds by electronic nose demonstrated that a clear discrimination of spice mixtures was possible.

## 12.5 CONCLUSIONS

Electronic noses were successfully applied for headspace analysis of spices. It was demonstrated in many investigations that electronic noses can contribute to the characterization of spices and spice mixtures in order to

- distinguish spices and spice mixtures
- differentiate by origin, growth seasons, and processing
- indicate adulteration from original
- detect mold infestation

Electronic noses can be used as a fast screening method to provide information about the product quality. However, it needs samples and methods for reference, careful training, and complex calibration to consider influencing and disturbing effects as well as the possible limitations of the instrumentation. The correlation to classical chemical analysis methods is always advisable. Machined olfaction methods are capable to support the sensory analysis; however, they cannot yet substitute them.

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# Chapter 13

# Tea and the Use of the Electronic Nose

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## 13.1 INTRODUCTION

Tea is an aromatic beverage prepared from the leaves of the tea plant, *Camellia sinensis*, and is the most widely consumed beverage in the world. Teas are classified into three major categories according to the manufacturing process, namely (1) unfermented green tea, (2) partially fermented Oolong tea, and (3) fully fermented black tea. The plantation of tea is highly season specific and climate dependent. Tea leaves are plucked from the field and brought to the processing plants where they undergo several processing stages and finished tea is produced. India is famous for production of black tea, whereas green tea is produced in countries like China and Japan. Other major tea-producing countries are Sri Lanka, Kenya, and Taiwan.

A review of the international standards published by the International Organization for Standardization (ISO) on tea reveals that the quality assessment methods of black tea by chemical analysis, using instruments such as gas chromatography–mass spectroscopy (GC–MS), high-performance liquid chromatography (HPLC), and other instruments, are quite established and have been in practice for several years. However, noninvasive, online, fast, low-cost, user-friendly, and practical deployable solutions for day-to-day use by the tea industries are still not available. The standards related to tea published by the ISO are given in Table 13.1 (www.iso.org).

In the tea industry, quality analysis of tea is carried out by expert tea tasters. Even though this is an established practice, the method is definitely not very accurate and depends upon the professional acumen, mood, and other personal factors of the tea taster. Also, chemical analysis techniques are expensive and time consuming and expert operators are required for the operation of the sophisticated instruments. The advent of the electronic nose has opened the way to a new analytical approach, which consists of an array of gas sensors with different selectivity patterns, signal handling, pattern recognition, and decision strategy; it has the capability to eliminate the drawbacks of the human tasters and the chemical analysis methods.

Considerable applications of the electronic nose have already been carried out on meat, grains, coffee, mushrooms, cheese, sugar, fish, beer, and other beverages, as well as on the quality evaluation of food-packaging material. "Electronic nose" applications have multiplied several times in recent years. The reliability of these applications is mainly based on the selectivity properties of the sensors composing the array and on the stability of their characteristics. In general, the electronic nose has wide applications in agriculture, biomedicine, automobiles, aerospace, environmental concerns, food, the military, pharmaceutics, explosives, and in various other fields (Bhattacharyya et al., 2008a; Brezmes et al., 2001; Di Natale et al., 2001; Boilot et al., 2000; Young et al., 2003; Capua et al., 2009).

## 13.2 TEA CHEMISTRY

The most important constituents of tea are catechins. These are colorless, odorless, soluble substances that have low molecular weight and constitute about 25% of total dry matter. These substances are oxidized by polyphenol oxidase or plant ferment during the fermentation process in tea manufacturing (Willson and Clifford, 1992). Also known as the oxidizable matter of tea, the catechins absorb oxygen with the help of enzymes. Once the oxygen is absorbed, catechins begin to form larger molecules through condensation, causing changes in color and some nonvolatile compounds such as theaflavins (TF) and thearubigins (TR) are produced (Obanda et al., 2001). The two major quality attributes of tea—strength and color—are dependent on the oxidizable matter present in the leaf. The next important constituents in tea leaves are enzymes. These enzymes play a vital part in the oxidation process as organic catalysts. These enzymes are of two types, namely, tea polyphenol oxidase and pectase. Tea polyphenol oxidase is the more important of the two. Another constituent of tea leaf is caffeine, which is 2.5–4.5% of the volume of tea leaves. This colorless and bitter compound is responsible for the stimulating properties of tea liquor. Briskness of brewed tea is largely dependent on the caffeine content of the leaves.

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**TABLE 13.1** List of ISO Standards Related to Tea

| ISO Standard | Details |
|---|---|
| ISO 1572:1980 | Tea—Preparation of ground sample of known dry matter content |
| ISO 1573:1980 | Tea—Determination of loss in mass at 103°C |
| ISO 1575:1987 | Tea—Determination of total ash |
| ISO 1576:1988 | Tea—Determination of water-soluble ash and water-insoluble ash |
| ISO 1577:1987 | Tea—Determination of acid-insoluble ash |
| ISO 1578:1975 | Tea—Determination of alkalinity of water-soluble ash |
| ISO 1839:1980 | Tea—Sampling |
| ISO 3103:1980 | Tea—Preparation of liquor for use in sensory tests |
| ISO 3720:1986 | Black tea—Definition and basic requirements |
| ISO 6078:1982 | Black tea—Vocabulary |
| ISO 6079:1990 | Instant tea in solid form—Specification |
| ISO 6770:1982 | Instant tea—Determination of free-flow and compacted bulk densities |
| ISO 7513:1990 | Instant tea in solid form—Determination of moisture content (loss in mass at 103°C) |
| ISO 7514:1990 | Instant tea in solid form—Determination of total ash |
| ISO 7516:1984 | Instant tea in solid form—Sampling |
| ISO 9768:1994 | Tea—Determination of water extract |
| ISO 9884-1:1994 | Tea sacks—Specification—Part 1: Reference sack for palletized and containerized transport of tea |
| ISO 9884-2:1999 | Tea sacks—Specification—Part 2: Performance specification for sacks for palletized and containerized transport of tea |
| ISO 10727:2002 | Tea and instant tea in solid form—Determination of caffeine content—Method using high-performance liquid chromatography |
| ISO 11286:2004 | Tea—Classification of grades by particle size analysis |
| ISO 14502-1:2005 | Determination of substances characteristic of green and black tea—Part 1: Content of total polyphenols in tea—Colorimetric method using Folin–Ciocalteu reagent |
| ISO 14502-2:2005 | Determination of substances characteristic of green and black tea—Part 2: Content of catechins in green tea—Method using high-performance liquid chromatography |
| ISO 15598:1999 | Tea—Determination of crude fibre content |

Pectine is another constituent and is a type of gelatizing substance found in the cell walls of tea leaf. It splits into pectic acid and methyl alcohol due to the action of pectase. Pectic acid helps to retain the quality of brewed tea.

The characteristic of odor of brewed tea depends upon certain aromatic substances that are volatile in nature. These substances, in turn, are made up of essential oils and a few others like amino acids. At the time of fermentation, orthoquinon combines with the amino acids and supplements the aroma of tea liquor.

Chemical composition of tea shoot varies with agroclimatic conditions, producing regions, agricultural practices, and the type of plants. As a result of extensive research, Tea Research Association (TRA), Tocklai, Assam, India, has identified chemical compounds available in black tea shoots (Bhuyan and Borah, 2001). Table 13.2 presents the important compounds responsible for the color, taste, and aroma of tea.

## 13.3 TRADITIONAL BLACK TEA QUALITY EVALUATION TECHNIQUES

This section presents some commonly used techniques employed for black tea quality assessment in tea industry:

1. *Chemical analysis methods*: These methods are very accurate and most scientific as high-end instruments that are used for analyzing the chemical composition of tea vapor and liquor. The instruments used for this purpose are GC, HPLC, and GC–MS. Although these instrumental methods of analysis yield high accuracy, they suffer from some distinctive shortcomings because the methods are time consuming and expensive and require elaborate sample preparation and expert manpower. Apart from these shortcomings, a major disadvantage of these methods is that these instruments can perform only a single attribute analysis at any given moment of time.

**TABLE 13.2** Biochemical Compounds Present in Tea

| Biochemical Compounds in Tea Responsible for Color | |
|---|---|
| **Compounds** | **Color** |
| Theaflavins | Yellowish brown |
| Thearubigins | Reddish brown |
| Flavonol glycosides | Light yellow |
| Pheophorbide | Brownish |
| Pheophytin | Blackish |
| Carotene | Yellow |
| **Biochemical Compounds in Tea Responsible for Taste** | |
| **Compounds** | **Taste** |
| Polyphenol | Astringent |
| Amino acids | Brothy |
| Caffeine | Bitter |
| Theaflavins | Astringent |
| Thearubigin | Ashy and slight astringent |
| **Biochemical Compounds in Tea Responsible for Aroma** | |
| **Compounds** | **Flavor** |
| Linalool, linalool oxide | Sweet |
| Geraniol, phenylacetaldehyde | Floral |
| Nerolidol, benzaldehyde, phenyl ethanol | Fruity |
| *Trans*-2-hexenal, *n*-hexanal, *cis*-3-hexenol, grassy, *β*-ionone | Fresh flavor |

These instruments are installed in big industrial houses and laboratories.

2. *Sensory evaluation*: In this case, an expert panel, called "tea tasters," is engaged to judge the quality of tea. They evaluate the finished tea against the attributes like appearance, taste, flavor, and aroma. In this evaluation process, they assign a score in the scale of 1 to 10 against each attribute of the sample under testing. Although this method is practiced in the tea industry, it has some unavoidable limitations like (1) subjectivity, (2) lack of repeatability, and (3) dependence on the mental state of the taster (fatigue, adaptation, etc.).

## 13.4 BLACK TEA PROCESSING—A BRIEF OVERVIEW

Black tea processing is performed through a few sequential operations: (1) plucking, (2) withering, (3) preconditioning, (4) cut-tear-curl (CTC), (5) fermentation, and (6) drying, as shown in Fig. 13.1.

The quality of the leaf depends upon the delivery and skill with which plucking is performed. Conventionally, only the bud with first and second leaves is plucked. The larger and coarser leaves are left on the bush. Plucked leaves are brought to the withering process where leaf moisture content is reduced by the blowing of air. The plucked tea leaves are spread over the withering troughs of a bed size approximately 20 × 100 ft. at a thickness of about 20–25 cm and air is blown through the tea leaves (Mahanta and Baruah, 1989). The cell structures of the withered leaves are disrupted by rotating vanes of specially designed machines in the preconditioning process. Thin membranes around the vacuole of the leaf cells are ruptured during the process, separating polyphenols and enzymes within the leaves. The CTC process comprises of cut, tear, and curl operations. In CTC machines, withered and preconditioned leaves are fed into a gap between two rollers having circumferential cutting edges and running at differential speeds. Physical parameters of finished black tea, like the dimension of tea particles and granular mix of finished bulk, may be modulated by varying the pitch of the rollers and the gap between the rollers. After the leaf cells have been ruptured by the previous processes, the fermentation process starts. The leaves are exposed to air and the oxidation process starts. The green leaves attain coppery brown color and a fragrant aroma starts to emanate. Thereafter, the leaves enter the drying process where they are subjected to a blast of hot air provided by means of a furnace. The factors that influence

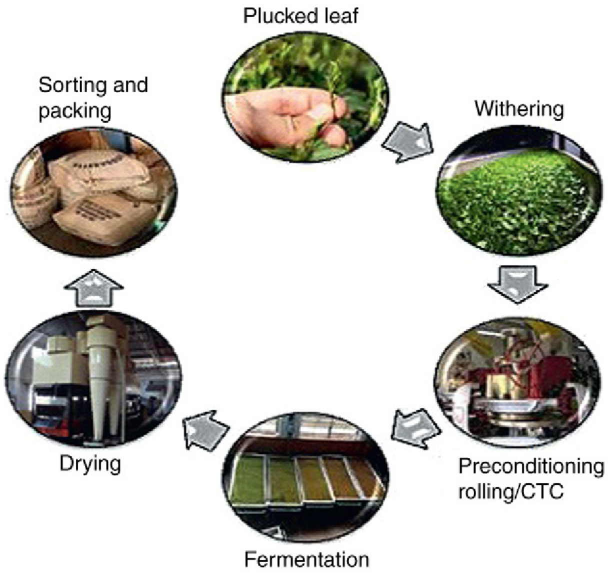

**FIGURE 13.1** Stages of black tea processing.

the process of drying are: (1) temperature of the air, (2) rate of feed, (3) run-through time, and (4) volume of air within the drying chamber.

Deactivation of enzymes, reduction of moisture, moderate development of black/brown appearance of tea, conversion of chlorophyll to pheophytin, degradation of lipids, and formation of some flavored components, and loss of some highly volatile components are a few of the numerous biochemical changes that occur during the drying process (Wickremasinghe et al., 1979).

### 13.4.1 Smell of Black Tea

The smell of tea depends upon certain volatile aromatic substances developed during the fermentation process. Fermentation is the most crucial of all the processes, since aroma and flavor are developed in this process through multidimensional biochemical pathways. These volatile substances are made up of essential oils and a few other factors such as amino acids (Co and Sanderson, 1970). During the process of fermentation, amino acids combine with orthoquinon, which is an oxidized form of catechin, and play the most important role for the black tea aroma.

The smell of teas in the fermentation process changes progressively as the process proceeds. Age-old empirical knowledge in black tea processing in India has established the fact that odor emanating in the fermentation process travels through two defined peaks of intense emission of volatiles with much reduced intensity of emission during intermediate spans during the fermentation time for black tea. Such smell peaks are popularly termed as "First Nose" and "Second Nose" in Indian tea industry parlance. Experienced floor supervisors can detect such distinct peaks of intense volatile emission by manually smelling the teas.

As soon as the "Second Nose" is detected, the supervisors call the end to the fermentation process. These so-called "First Nose" and "Second Nose" peaks are not only very sharp and prominent, but also very much short lived (Motoda, 1979). It is quite possible that the supervisors may not always be able to detect such short-lived bursts of odor peaks by their olfactory senses. In the event of such inadvertent mistakes on the part of floor operators/ supervisors, the tea produced will be either underfermented or overfermented. Such an age-old process, though empirical, is being practised by the Indian tea industries from time immemorial. Such practices definitely are highly subjective, unreliable, and prone to human mistakes and thus often lead to production of inferior quality tea due to over- or underfermentation. The black tea fermentation monitoring process and optimum fermentation time detection using electronic nose technology was reported by Bhattacharya et al. (2007a,b, 2008b).

## 13.5 LITERATURE SURVEY ON ELECTRONIC NOSE-BASED TEA QUALITY EVALUATION

So far, several research groups have demonstrated the applicability of electronic nose technology for tea quality evaluation. The applications may be classified into two groups: (1) analysis on finished tea and (2) monitoring during the fermentation stage. This section presents the research reports on tea quality evaluation employing the electronic nose.

Pioneering work had been done by Dutta et al. (2003) in the field of tea using the electronic nose, where the efficacy of electronic nose systems in classifying black tea aroma and flavor in different processing stages was established. For data analysis, fuzzy C-means (FCM), principal component analysis (PCA), self-organizing map (SOM), learning vector quantization (LVQ), multilayer perceptron (MLP), and other neural network topologies are explored in this work. Several researchers attempted to classify different grades of tea employing electronic nose (Bhattacharyya et al., 2004; Kashwan and Bhuyan, 2005; Yang et al., 2006; Yu and Wang, 2007; Yu et al., 2008). Bhattacharyya et al. (2008a,b) in two different papers presented the design of the electronic nose with commercial metal oxide sensor (MOS) sensors for black tea quality discrimination and a method for enhancement of sensitivity of measurement employing an illumination heating and raking process. Yu et al. (2009a,b) studied tea storage time and tea grade for green tea, using the MOS-based electronic nose. Evaluation of a particular flavor of green tea was studied using the electronic nose in Yang et al. (2009). In another study of black tea classification employing the electronic nose (Tudu et al., 2009a), an incremental radial basis function of the neural network was proposed for data analysis. The incremental learning ability can be of great benefit by automatically including the newly presented patterns in the training data set without affecting the class integrity of the previously trained system. For application in black tea grade discrimination (Tudu et al., 2009b), another attempt was made to correlate the multisensor aroma pattern of the electronic nose with a sensory panel (tea tasters) evaluation, and for classifying, an incremental learning fuzzy model was proposed. The algorithm was tested in some tea gardens of northeast India, and the results were presented. In another study (Sipos et al., 2011), the performance of sensory panel was evaluated using two different approaches, namely, gravity center area/perimeter (GCAP) and compare ranks with random numbers (CRRN). In a study (Zhang et al., 2011), the capacity of an electronic nose (E-nose, PEN2) to classify tea quality grades was investigated. Three groups of tea with different quality grades were harvested at different times. Principal component analysis (PCA) and artificial neural network (ANN) were employed for data analysis. These results indicate that the electronic nose could be successfully used for the detection of teas of different quality grades and ages. In order to optimize the performance of the electronic nose and to make it application specific, a rough set theory was used for selecting the most relevant and nonredundant feature from data sets (Bag et al., 2011). The performance of the electronic nose was further enhanced (Kaur et al., 2012) using a dynamic social impact theory-based optimizer (SITO) along with PCA and support vector machine (SVM).The performance of SVM was further validated (Chen et al., 2011) in order to compare four grades of green tea. In another study (Qin et al., 2013), MOS sensor-based electronic nose was used to distinguish the differences between different grades of green and black teas. With a portable electronic nose, the classification of three different fermentation degrees of tea (ie, green tea, black tea, and Oolong tea) was studied (Chen et al., 2013). In a recent study (Mirasoli et al., 2014), the electronic nose was used for evaluation of change in green tea quality during long-term storage and the electronic nose was found to classify correctly unknown samples as “aged” or “not aged.” In another recent work (Torri et al., 2014), an electronic nose was employed to study the volatile emissions of leaf samples belonging to the basic Chinese teas (white, yellow, green, oolong, black, and Pu-erh) with those of their respective infusions and an interesting observation was reported that the leaf aroma was not transferred fully into the beverage.

As already mentioned, fermentation process in tea manufacturing plays the key role in determining the quality of finished tea. At the time of fermentation, the grassy smell of the leaves changes to the floral smell due to some complex chain of biochemical reactions inside the tea leaf and the greenish color changes into the coppery brown. We mention here a few research reports on monitoring the aroma during the fermentation process with the electronic nose.

The monitoring of volatile components of the black tea during the fermentation process was studied with a MOS sensor array-based electronic nose (Bhattacharyya et al., 2007a). An electronic nose was used for the detection of optimum fermentation time during tea processing (Bhattacharyya et al., 2007b). A study on real-time smell monitoring of black tea during the fermentation process using an electronic nose was performed for prediction of the correct fermentation time (Bhattacharyya et al., 2008c). In this study, along with optimum fermentation time, the detection of the existence of different smell stages during the fermentation runs of black tea processing was studied.

Presently, research is going on to fabricate sensors that would be more sensitive and selective to the volatile organic and aromatic compounds of tea. In this direction, there are few research reports on coated quartz crystal microbalance (QCM) sensors for estimating the most important aroma producing compounds in tea—linalool (Sharma et al., 2014) and geraniol (Sharma et al., 2015). The coating materials are selected or synthesized in order to achieve better sensitivity and selectivity toward these tea chemicals.

The research reports previously mentioned indicates that there are quite a few groups in different parts of the world working in the area of electronic nose technology exclusively for tea. Their findings show the potential and promise of the instrument to be used in the tea production units well as in the tea-tasting centers where aroma plays a crucial role either in controlling some process parameters or estimating the quality of the product. Table 13.3

**TABLE 13.3 Major Research Publications on Application of Electronic Nose for Tea (2003–2014)**

| Sample | Type of Study | Sensor System | Data Processing Algorithm | Year of Publication | References |
|---|---|---|---|---|---|
| Black tea | Discrimination between the flavors of different tea samples | MOS | PCA, FCM, SOM, MLP, LVQ, RBF, PNN | 2003 | Dutta et al. (2003) |
| Black tea | Characterization and classification of six clonal varieties of orthodox black tea | MOS | PCA, BP-MLP | 2004 | Bhattacharyya et al. (2004) |
| Black tea | Discrimination and classification of electronic nose response data for different flavors of tea and spice | MOS | PCA, MLP, RBF, LVQ | 2005 | Kashwan and Bhuyan, (2005) |
| Tea | Classification of tea |  | New bionic olfactory neural network model based on the KIII set in the K-set hierarchy | 2006 | Yang et al. (2006) |
| Green tea | Classification of tea quality grade with four LongJing green tea | MOS | BP–MLP, PCA, linear discriminant analysis (LDA) | 2007 | Yu and Wang (2007) |
| Black tea | Estimation of optimum fermentation time | MOS | 2-Norm method, Mahalanobis distance method (MDM) | 2007 | Bhattacharyya et al. (2007a) |
| Black tea | Monitoring the volatile components of the black tea during fermentation process and detection of the optimum fermentation time on the basis of peaks in the sensor outputs | MOS | PCA, SVD | 2007 | Bhattacharyya et al. (2007b) |
| Black tea | Smell peak prediction during fermentation | MOS | SOM, time delay neural network (TDNN) | 2008 | Bhattacharyya et al. (2008c) |
| Green tea | Gradation of different green tea samples | MOS | PCA, LDA | 2008 | Yu et al. (2008) |
| Black tea | Selection of appropriate sensors for black tea aroma and development of a taster-specific computational model for objective prediction of tea quality scores | MOS | PCA, BP–MLP, PNN, RBF | 2008 | Bhattacharyya et al. (2008a) |
| Black tea | Performance of electronic nose improved with illumination heating and physical raking of the sample | MOS | BP–MLP, PNN | 2008 | Bhattacharyya et al. (2008b) |
| Black Tea | Quality evaluation of black tea | MOS | Incremental RBF network | 2009 | Tudu et al. (2009a) |
| Black Tea | Classification of black tea | MOS | Incremental Fuzzy Logic | 2009 | Tudu et al. (2009b) |
| Green tea | Identification of tea storage time | MOS | PCA, LDA, BP–MLP | 2009 | Yu et al. (2009a) |
| Green tea | Identification of coumarin-enriched Japanese green teas and evaluation of their particular flavor | MOS | PCA, cluster analysis (CA) | 2009 | Yang et al. (2009) |
| Green tea | Quality grade identification | MOS | PCA, LDA, BP–MLP | 2009 | Yu et al. (2009b) |
| Sri Lankan black tea | Evaluation of the performance of a sensory panel using a novel method with electronic nose | MOS | PCA, LDA, PLS, SOM | 2011 | Sipos et al. (2011) |
| Green tea | Detection of teas of different quality grades and ages | PEN-2 | PCA, ANN | 2011 | Zhang et al. (2011) |

| Sample | Type of Study | Sensor System | Data Processing Algorithm | Year of Publication | References |
|---|---|---|---|---|---|
| Black Tea | Optimization of sensor in an array of electronic nose | MOS | Rough set theory | 2011 | Bag et al. (2011) |
| Green tea | Discrimination of four grades of green tea | | PCA, *k*-nearest neighbor (*k*-NN), artificial neural network (ANN), SVM | 2011 | Chen et al. (2011) |
| Black tea | Identifying the optimum time intervals of the EN sensor array response | MOS | SITO and moving window time slicing in conjunction with PCA and SVM | 2012 | Kaur et al. (2012) |
| Green and black tea | To distinguish the difference among different grade teas | MOS | Partial least square (PLS) regression | 2013 | Qin et al. (2013) |
| Three different fermentation degrees of tea | Classification of tea category according to different fermentation degrees | Porphyrins, metalloporphyrin materials | LDA | 2013 | Chen et al. (2013) |
| Green tea | Evaluation of the quality changes in commercial green tea leaves during a long-term storage | MOS | PCA, LDA | 2014 | Mirasoli et al. (2014) |
| Different categories of tea | Differentiate between different categories of tea using electronic nose | MOS | PCA | 2014 | Torri et al. (2014) |
| Black tea | Detection of linalool in black tea | QCM | Correlated with GC–MS analysis | 2014 | Sharma et al. (2014) |
| Black tea | Detection of geraniol in black tea | QCM | Correlated with GC–MS analysis | 2015 | Sharma et al. (2015) |

summarizes the research reports on the use of the electronic nose for tea applications.

## 13.6 CASE STUDY

A case study with the development and customization of an electronic nose is presented in this section (Bhattacharyya et al., 2008a). The instrument uses Figaro gas sensors for tea quality estimation. The following considerations were the basic guiding philosophy for development of the system:

1. The system should be user friendly and easy to operate so that individuals in the tea industry can use the system.
2. The modular approach can be employed for hardware and software design for ease of integration.

The instrument consists of (1) a sensor array, (2) an odor delivery system using a micropump, mass flow controller, and solenoid valves, (3) PC-based data acquisition, and (4) olfaction software.

### 13.6.1 Selection of Sensors

The most important part of electronic olfaction process is odor capture and associated sensor technology. Any sensor that responds reversibly to chemicals in the gas or vapor phase has the potential to be a part in an array of sensors in an electronic nose. Therefore, in this research of tea aroma detection, an array of MOS sensors from Figaro, Japan (www.fiagrosensor.com) was used in the experimental setup. MOS have a number of features like high sensitivity, high stability, reliability over a long period, and very good reversibility. The sensing element for such sensors is tin dioxide ($SnO_2$), which has a low conductivity in clean air. In the presence of detectable vapor, the conductivity of the sensor increases depending on the concentration of odor molecules in the vapor. The output of the sensors is processed by a signal conditioning circuit for signal amplification, buffering, and signal conversion.

In order to select the appropriate sensors adequately sensitive to black tea aroma, the following major flavor compounds in black tea, as given in Table 13.3, were collected from Tea Research Association, India. The sensors considered for black tea classification were TGS-816, TGS-823, TGS-831, TGS-832, TGS-2600, TGS-2610, TGS-2611, and TGS-2620 of Figaro Engineering Inc. Seven important volatile organic compounds of black tea of concentration 50 ppm were used to excite each of the MOS sensors in an experiment. The changes in sensor resistance $\Delta R_S/R_S$ were measured very precisely.

Although sensor selection was based on sensitivity analysis with a handful of major aroma determinants, there would be contribution of other volatiles on the sensor responses when exposed to tea flavors. But our specific objective in this study was to track the overall effect of these volatiles on the sensor array and investigate the correlation of the multisensor output data with sensory evaluation.

Responses of all the sensors to the chemicals listed in Table 13.4 are graphically shown in Fig. 13.2. The details of the electronic nose setup along with the pattern recognition unit is presented in Bhattacharyya et al. (2008a).

The final sensor set, therefore, comprises of the five sensors—TGS-823, TGS-832, TGS-2600, TGS-2610, and TGS-2611.

During the experimental studies with the electronic nose, it has been observed that brewed tea liquor could not be used due to presence of water vapor in the headspace. Therefore, only dry tea leaves are used in the sample holder for headspace generation and sampling. But the

**TABLE 13.4** $\Delta R_S$ Values for Individual Sensors When Exposed to Specific Tea Chemicals

| Sl. No. | Names of Chemicals | TGS 2610 (1) | TGS 2620 (2) | TGS 2611 (3) | TGS 2600 (4) | TGS 816 (5) | TGS 831 (6) | TGS 832 (7) | TGS 823 (8) |
|---|---|---|---|---|---|---|---|---|---|
| 1. | 2-Phenyl-ethanol | 0.21 | 0.05 | 0.24 | 0.26 | 0.02 | 0.01 | 0.35 | 0.39 |
| 2. | Benzaldehyde | 0.03 | 0.01 | 0.23 | 0.17 | 0.03 | 0.01 | 0.19 | 0.51 |
| 3. | β-Ionone | 0.06 | 0.04 | 0.42 | 0.40 | 0.03 | 0.03 | 0.78 | 0.74 |
| 4. | Geraniol | 0.04 | 0.05 | 0.16 | 0.13 | 0.01 | 0.01 | 0.22 | 0.39 |
| 5. | Linalool | 0.23 | 0.07 | 0.59 | 0.49 | 0.09 | 0.06 | 0.95 | 0.87 |
| 6. | Linalool oxide | 0.15 | 0.06 | 0.62 | 0.58 | 0.02 | 0.07 | 0.97 | 0.89 |
| 7. | Terpeniol | 0.07 | 0.04 | 0.41 | 0.36 | 0.02 | 0.03 | 0.65 | 0.74 |

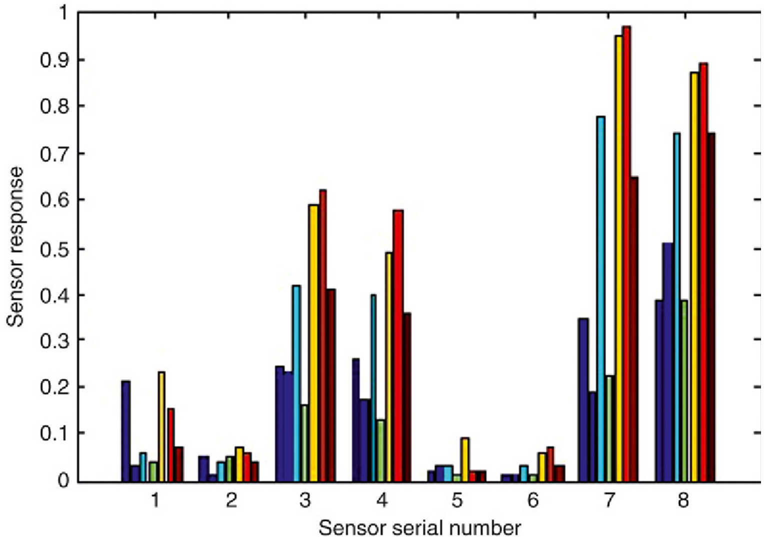

**FIGURE 13.2** Sensor's response to individual chemicals.

volatile emission from dry tea being considerably low, sensor outputs have been observed to be significantly small. On the other hand, tea scientists have established that optimum volatile emission takes place from the tea at around 60°C ± 5°C. In fact, good flavor of brewed tea is caused due to rise in temperature of the tea by addition of boiling water to it. To resolve this conflicting requirement, a novel method of heating of the sample under test using optical energy from commonly used miniature halogen lamps has been developed along with simple motorized mechanical agitation system of tea samples within the sample holder.

With this setup, extensive experimentation had been carried for tea quality, both for finished tea and in fermentation level. We present here the results of classification of 48 samples of 4 different grades collected from tea gardens of north and northeast India, namely, Singbulli, MinFTGFOP, Sungama, and Chamong. For each sample, 10 replicated measurements were taken. For our experiments, one expert tea taster was deputed to provide a taster's mark to the samples in a scale of 1–10 and the range of the scores assigned for these samples were from 5 to 9.

The experimental conditions are given as follows:

- Amount of each sample = 40 g
- Temperature = 60°C ± 3°C
- Headspace generation time = 30 s
- Collection time = 100 s
- Purging time = 100 s
- Airflow rate = 5 mL/s

### 13.6.2 Results and Discussions

As a first step to identify underlying clusters in the electronic nose signatures, the data obtained were analyzed using principal component analysis (PCA). In view of the capability of neural networks to learn input–output relation from a training data set, the neural network was chosen for tea classification and three topologies, for example, the back-propagation multilayer perceptron (BP–MLP) method, the radial basis function (RBF), and the probabilistic neural network (PNN) were considered.

The PCA plot in Fig. 13.3 clearly points out the existence of distinct clusters of electronic nose patterns with respect to the tea tasters' scores.

Further, the neural network analysis was carried out with 480 observations (48 samples × 10 replicated measurements). Of these patterns, 60% from each class have been used for training, and the remaining 40% of these patterns were used for testing. For these 40% samples, the predicted quality scores by the trained neural network model were compared with the aroma scores of the tea tasters. It was observed that the percentage accuracies of prediction with the three topologies of the neural network (BP–MLP, RBF, and PNN) were 84.21, 83.11, and 79.43%, which clearly

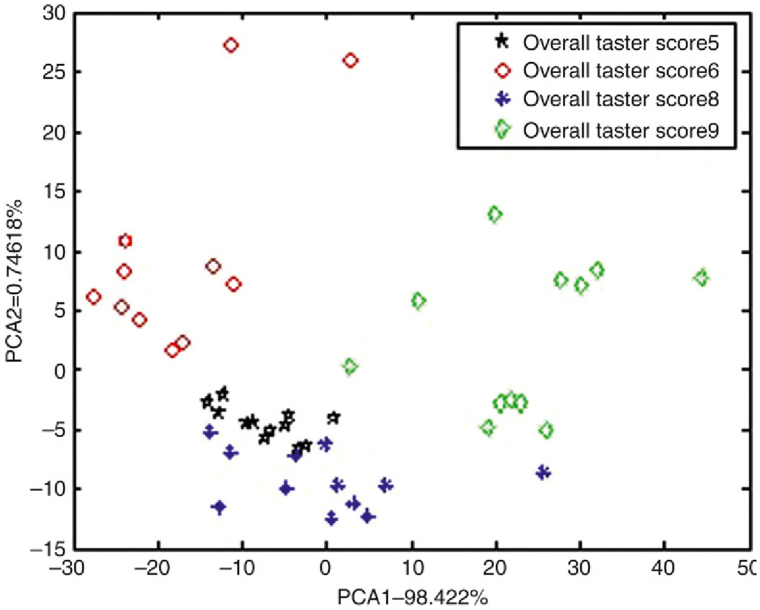

**FIGURE 13.3** PCA plot of four varieties of tea sample with electronic nose.

establishes the efficacy of the electronic nose instrument (Banerjee et al., 2012).

## 13.7 CONCLUSIONS

The focus of this chapter has been on tea samples with the electronic nose and the research findings so far are quite encouraging even with the nonspecific MOS sensors in the array. There is a vast scope of research as very little has been done for this aromatic agroproduct. Opportunities prevail in the development of new sensors with more specificity as well as in the overall system design. During tea processing, aroma plays a very important role in optimizing different parameters during the processing of the leaves and electronic nose, if tuned properly for these applications may usher a new paradigm in monitoring and controlling the tea processes. Although tea is a flavored compound, aroma, taste, and appearance are other major attributes for its quality. So, along with the electronic nose, application of the electronic tongue and the electronic eye should give better quality judgment for tea. A few research publications are already published in this direction.

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## Chapter 14

# Wine Applications With Electronic Noses

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## 14.1 INTRODUCTION

Wine is one of the most complex alcoholic beverages with more than 1000 of volatile components identified in its headspace ranging from a few parts per billion to a few percents in weight, mainly alcohols, esters, ketones, acids, ethers, aldehydes, terpenes, lactones, sulfur-, nitrogen-, carbonyl-, phenolic-compounds. Hence, the feature extraction procedure results elaborated to qualitatively and quantitatively assess the wine aroma profile. Due to high economic value of the wine-product for some worldwide typical geographical areas and annexed sociocultural reasons, the development of analytical methods and pattern recognition systems for wines' classification is extremely important, mainly for the assignment of a trademark such as protected designation of origin (PDO), controlled denomination of origin (CDO), and protected geographic indication (PGI) for quality wines. In this context, useful analytical systems coupled to pattern recognition methods serve for wines' identification and, consequently, to protect the trademarked quality wines and to prevent their illegal adulteration.

The detection of aroma and the quality control of wine can be assessed by different analytical methods for the identification of the organoleptic properties of the products. In fact, the classical methods of chemical analysis such as gas and liquid chromatography, mass spectrometry, nuclear magnetic resonance, and spectrophotometry are highly reliable and suitable for these purposes, but these analytical techniques are of high cost, long processability, and low in situ and online measurableness.

The human nose is currently used commercially to test a diverse range of products. Highly skilled, trained human panels have been used to evaluate the odors produced from food products, such as the wine, in order to determine its quality (Horrillo et al., 2007). The practical application of human nose as a smell assessment instrument is severely limited by the fact that our sense of smell is subjective, gets tired easily, and is therefore difficult to use. Consequently, there is considerable need for an instrument that could mimic the human sense of smell and be used in routine industrial applications. E-noses are attractive for a number of significant features: the relatively fast assessment of headspace, the qualitative representation or signature of an aroma, and the use of cheap sensors to be integrated in production processes. Despite these features, there are still relatively few applications of e-noses adopted in the wine industry. This could be attributed to difficulties in robustness, selectivity, and reproducibility of the sensors and to the need for pattern recognition algorithms. Nonetheless, the use of e-noses is rapidly expanding and notable achievements, relevant for the food industry, have been achieved in the last few years. Furthermore, this progress coincides with an increased understanding of the biological mechanisms behind the human olfactory system (Loutfi et al., 2015). However, there is much research still to be done especially with regard to new materials and sensors technology, data processing, interpretation of results, and validation studies (Peris and Escuder-Gilabert, 2009).

The main purposes of this chapter are: to offer the reader a review of the different technologies involved the e-nose field applied to the wine industry and to serve as a guide for future applications and development since the main difficulties and future perspectives in this field are discussed.

## 14.2 ELECTRONIC NOSES OPERATION

An accepted definition of an e-nose was given by Gardner in 1999 and restricts the term to those types of sensor array systems that are specifically used to sense odorous molecules in an analogous manner to the human nose. However, the architecture of an e-nose has much in common with multisensor systems designed for the detection and quantification of individual components in a simple gas or vapor mixture. A simple flowchart of the typical structure of an e-nose for wine applications is shown in Fig. 14.1. It generally consists of an aroma extraction technique, which switches the reference air and the sample; an array of chemical sensors which transforms the aroma into electrical signals; an instrumentation system to measure the sensor signal; and a pattern recognition system to identify and classify the aroma of the measured samples in the

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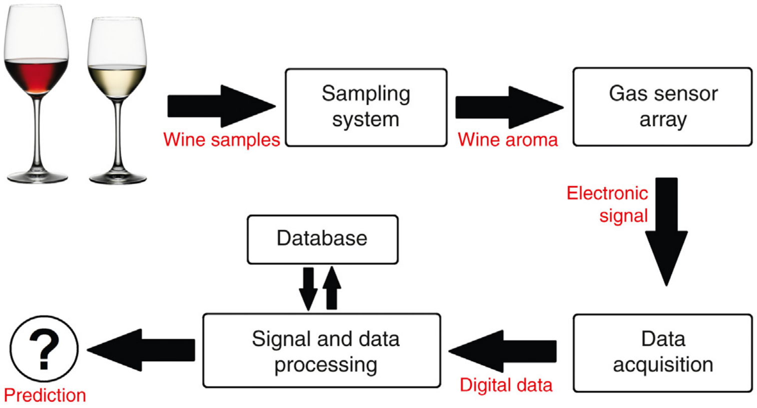

**FIGURE 14.1** **Block diagram of an e-nose for wine discrimination.**

classes previously learned when using supervised learning or perform by itself the classification in unknown classes (Horrillo et al., 2007).

It uses currently a number of individual sensors (typically 4–100) whose selectivities toward different molecules' overlap. The response from a chemical sensor is usually measured as the change of some physical parameter, for example, conductivity, frequency, or current. The response times for these devices range from seconds up to a few minutes. By teaching a computer (or hardware) to recognize different patterns, it should now be able to classify the wine aroma belonging to the different classes of learned aromas or patterns.

### 14.2.1 Sampling Methods

The aroma extraction system or sampling method carries the aromatic compounds from the wine samples to the sensor chamber. Several aroma extraction techniques are usually used for e-noses in wine applications (Lozano et al., 2007b). The most common are static headspace (HS) (Penza and Cassano, 2004), purge and trap (P&T) (Santos et al., 2004), and solid-phase microextraction (SPME) (Guadarrama et al., 2001; Lozano et al., 2008b), among others.

In the HS method, a thermodynamic equilibrium is allowed between the liquid sample and its vapor phase and then it is extracted and transferred to the sensors by a constant flow of an inert gas to prevent wine oxidation. HS is widely used for its simplicity and reproducibility. The main drawback of this method is the extraction of high amounts of water and ethanol that can interfere with the sensor response.

The P&T method is based on the transport of volatiles compound to a trap by means of an inert gas and the subsequent thermal desorption. This method has the advantage of increasing the selectivity and sensitivity toward wine compounds, thus increasing the discrimination capability of the e-nose.

The SPME method consists of the extraction of analytes from the matrix through the adsorption on a silica fiber covered by a sorbent material. Desorption is achieved by temperature or by organic solvents.

Comparisons between methods are a further subject of study. Two types of purging, trapping methods and four types solid-phase microextraction methods were compared in Lozano et al. (2008a,b). Aside from the sample handling, the sample itself may be preprocessed such as in the analysis of the quality of wine and beer; the preprocessing procedure of dehydration and dealcoholization helped the e-nose to classify aromas to a better extent (Ragazzo-Sanchez et al., 2005).

### 14.2.2 Sensors

The core of an e-nose for wine applications consists of an array of gas sensors useful for the analysis of the aroma of wine samples. Most common are conductive sensors (Smyth and Cozzolino, 2013), to which two types of materials are commonly used: metal oxides (MOX) (Santos et al., 2004) and conducting polymers (CP) (Guadarrama et al., 2001). Apart from conductive sensors, gas detection has also been done using optical sensors (Elosua et al., 2012) and gravimetric sensors, such as quartz microbalance (QMB) sensors (Di Natale et al., 2004) and surface acoustic wave sensors (SAW) (García et al., 2006).

Semiconductor metal-oxide-based gas sensors have been studied for many years; despite this, further research is ongoing mainly to improve their sensitivity, selectivity, and stability. Sputtering, thermal vacuum deposition, chemical vapor deposition (CVD), and sol–gel process are the most widely used deposition techniques for the sensitive layers. They are deposited either as a thick or thin film over different types of substrates, mainly ceramic or silicon. Although they are strongly affected by water and ethanol, coupling with selective extraction techniques, using calibration methods and careful design allow e-noses based on them a great discrimination power.

Conducting polymer gas sensors exhibit interesting properties that make them useful for gas sensors: room temperature operation, easy to prepare, and quick response, among others. They experiment changes in their electrical resistance when exposed to different volatile species. Most used sensors in wine discrimination are based on the following conducting polymers: polypyrrole, poly-*N*-methylpyrrole, polyaniline, and polythiophene, and their derivatives. In spite of some promising perspectives, these sensors lack specificity, show a limited reproducibility, and display a marked cross-sensitivity to water vapor.

Quartz crystal microbalance sensors essentially weigh the amount of gas or vapor interacting with a sensing layer coated on a microbalance. SAW sensors work in a similar way; the vapor is sorbed by the sensitive layer, resulting in a mass increase which modifies the surface wave velocity in the device. Several materials are used as sensitive layers in both cases. The ones most used are phthalocyanines, cyclodextrins, organometallic compounds, and rubber polymers as polyepichlorohydrin (PECH), polyetherurethane (PEUT), polybutadiene (PBD), and polydimethylsiloxane (PDMS).

### 14.2.3 Data Acquisition

The instrumentation system of an e-nose measures the sensor chemical signals and converts them to electrical signals amplifying and conditioning them, if necessary. The signal must be converted into a digital format to be processed by a computer for further signal and data analysis. Several methods are used for acquiring the data for use in further blocks: microprocessors and microcontrollers combined with A/D converters, digital multimeters and counters combined with multiplexers connected to computers and data acquisition cards. The choice of the technology to use depends on the requirements of size, power, type of sensors used, and application of the designed e-nose. One of the critical features of the data acquisition system is the resolution obtained in the measurements that depends directly on the resolution selected in the analog to digital converters used.

### 14.2.4 Data Processing

The multivariate information obtained by the sensor array can be sent to a display so a human can read that information and do an action or an analysis. Also that information, that is an electronic fingerprint of the volatile compound measured, can be sent to a computer to perform an automated analysis and emulate the human nose. This automated analysis usually comes from methods of statistical pattern recognition, neural networks, and chemometrics. Data processing systems are composed by several stages of processing multivariate data. In the first, the sensor data are preprocessed. In this sense, the data curves are usually smoothed, drift is compensated, outliers are eliminated, and also extracting of descriptive parameters can be done in this phase. In the second stage, an extraction or a selection of the features that will be used by the pattern recognizing method is done. In the third part, a classifier is used to decide to which class the measured sample belongs. The final stage is to validate the model with additional data to estimate its accuracy. Choosing an inappropriate classification algorithm could result in poor results, either because the algorithm lacks sufficient plasticity to model nonlinear data, or because a highly plastic model was overtrained on an insufficient number of training data. A recent manuscript (Marco and Gutierrez-Galvez, 2012) reviews the advances made in recent years in signal and data processing for machine olfaction and chemical sensing.

### 14.2.5 Electronic Noses for Wine Discrimination

An e-nose for wine discrimination must collect several general requirements. First, the choice of a suitable extraction procedure to qualitatively and quantitatively represent the wine original aroma. Moreover, the samples management must be done avoiding wine evolution. In other words, it can be difficult to obtain a representative extract of the wine which has not been altered or degraded in any way. One of the main problems that researchers have to face when they are designing an e-nose for wine is the influence of water and ethanol. In this sense, it is preferable to use sampling systems that could eliminate this influence or sensors with low sensitivity to them or those saturated at low concentrations. An additional problem is the presence of sulfur compounds in wine, which cause poisoning of the sensors and an important degradation in their characteristics. Moreover, sensors of the array and the measurement parameters should be optimized in order to offer the best discrimination of the samples to analyze. Concerning the instrumentation, the acquisition of the sensor signals with a high signal-to-noise ratio and resolution is a key aspect to obtain an optimal data for further analysis. Another important aspect related with the electronics is the control of the parameters of the e-nose: flows, temperatures, reference voltages, and so forth, should be carefully controlled with the minimum error in order not to alter the environment of measurement. Finally, the successful design of a data processing system requires a careful consideration of the various issues involved in processing multivariate data. Special attention must be paid to drift compensation algorithms, recalibration procedures, and robust classifiers when they are applied to the wine field.

As an example of a specific development of an e-nose for wine discrimination is presented in (Lozano et al., 2014). It shows the development of an in situ and online e-nose installed in a wine cellar of Madrid O.D. for the continuous measurement of the wine evolution (Fig. 14.2). The system has a novel sampling method that extracts the aroma directly from the tanks where wine is stored; and it automatically carries the volatile compounds to the sensor cell with the advantage of making continuous monitoring of the wine without the need of taking samples. The whole system

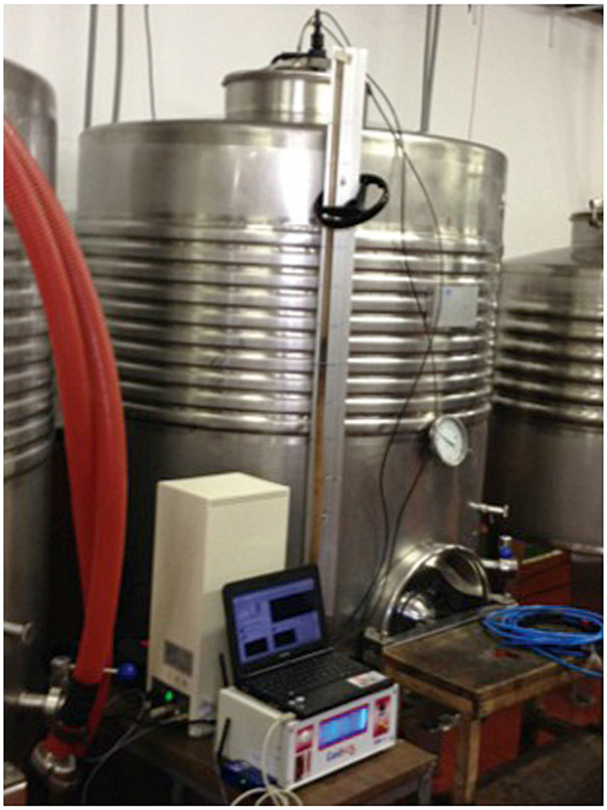

FIGURE 14.2 E-nose for wine discrimination installed in an experimental wine cellar.

is fully automated and controlled by the computer and can be supervised on the Internet. This system was also able to differentiate both wines and to detect the controlled alterations produced in the same ones (oxidization, correction of volatile acidity, pH, etc.) along 9 months. Correlation coefficients near to 1 are obtained in the prediction of several VOCs concentrations determined by GC–MS. This system could contribute for detecting off-odors and warning the wine expert to correct it as soon as possible, preventing the wine spoilage and improving its final quality.

There are some commercial general-purpose e-noses that have been used in wine applications. Some examples are: A32S Aromascan (Pinheiro et al., 2005); HERACLES (Yu et al., 2014); FOX 2000, 3000, and 4000 (Ragazzo-Sanchez et al., 2006, 2008; Berna et al., 2008); PEN-3 (Macías et al., 2013); Cyranose 320 (Ragazzo-Sanchez et al., 2005); and Znose (Duarte-Mermoud and Beltran, 2009).

## 14.3 WINE APPLICATIONS OF ELECTRONIC NOSES

Since the development of the "so-called" e-noses, the amount of publications in the area of artificial olfaction is more than 12,000 articles and they have been applied to several fields summarized in several review papers (Smyth and Cozzolino, 2013; Loutfi et al., 2015) and books (Pearce et al., 2006; Gardner and Bartlett, 1999). As a general rule, the discrimination of the wines is not an easy task due to the complexity and heterogeneity of its headspace. However, the classification of the wines is very important because of high economic value of the wine-product, to protect the quality wines, to prevent illegal adulteration of wines, to guarantee the wine quality in import–export market, and to control beverage processing. Huge quantities of applications of e-noses have been described for wine-producing industry and were summarized in Table 14.1 and some of them are described in the following sections.

### 14.3.1 Wine Aroma and Wine Classification

Wine is a very complex commercial product, which can vary greatly in aroma and flavor according to the large possible variations in its production. The formation and transformation of organic acids at must fermentation and wine production are of great importance in wine making. Biochemical processes caused by yeast enzymes are significant to achieve better quality of wine. Organic acids also protect wine against bacterial diseases. However, high content of some acids badly influences wine flavor.

The qualitative and quantitative analyses of the most dominant aromas and flavors evolve from the headspace of wine,

**TABLE 14.1** Electronic Noses and Their Wine Applications

| Application | Sensor Technology[a] | Number of Sensors | Data Processing Algorithm | References |
|---|---|---|---|---|
| Oxygen level, phenolic content in red wines | MOX | 14 | PLS-DA | Rodriguez-Mendez et al. (2014) |
| Wine compounds solution | MOX | 2 | PCA | Wongchoosuk et al. (2009) |
| | CP (Aromascan) | 32 | PCA | Pinheiro et al. (2005) |
| Red wine aging | MOX | 10 | PCA, LDA, CA | Wei et al. (2014) |
| | MOX | 9 | PCA, Tucker3 | Prieto et al. (2012) |
| | MOX | 4 | PLS-DA | Apetrei et al. (2012) |
| | MOX | 16 | PCA, RBFNN | Santos et al. (2011) |
| | MOX | 16 | PCA, RBFNN | Lozano et al. (2008a) |
| | FID (HERACLES) | 1 | PCA, DA | Yu et al. (2014) |
| Acetic acid in wine | MOX (PEN-3) | 10 | PCA, MLP | Macías et al. (2013) |
| | MOX | 4 | PCA, RBFNN | Lozano et al. (2011) |
| Influence of wine bottle closures | MOX | 15 | PCA, PLS-DA | Prieto et al. (2011) |
| Wine spoilage, off -flavors | Potentiometric | 5 | PCA, CLA | Gil-Sánchez et al. (2011) |
| | MOX (FOX 3000) | 12 | PCA, CLA | Cabañes et al. (2009) |
| | MOX (FOX 4000) | 18 | PCA, DFA | Ragazzo-Sanchez et al. (2009) |
| | MOX (FOX 3000), MS | 12, 1 | PLS | Berna et al. (2008) |
| | MS | 1 | PCA, PLS | Cynkar et al. (2007) |
| | | 1 | PLS | Martí et al. (2003) |
| Discrimination between wines with special grape treatments (dried, sprayed, …) | CP (Cyranose) | 32 | PCA, CDA | Zoecklein et al. (2011) |
| | QMB (QMB6) | 6 | ANOVA, CDA, PCA | Martin et al. (2008) |
| | MOX (FOX 4000) | 18 | PCA | Ragazzo-Sanchez et al. (2005) |
| | CP (Cyranose) | 32 | ANOVA, PCA | Devarajan et al. (2011) |
| | QMB | 8 | | Lopez de Lerma et al. (2012) |
| Alcoholic fermentation | MOX | 10 | PCA | Buratti et al. (2011) |
| Wine classification | MS | 1 | PCA, PLS | Cozzolino et al. (2010) |
| | MOX | 16 | PCA, PNN | Santos et al. (2004) |
| | SAW | 8 | DFA, PCA | McKellar et al. (2005) |
| | MOX (FOX 3000) | 12 | PCA | Martí et al. (2004) |
| | MS | 1 | PCA, PLS, ICA | Di Natale et al. (2004) |
| | QMB | 8 | PCA, BPANN | Penza and Cassano (2004) |
| | MOX | 4 | PCA, PLS, RSR | Capone et al. (2013) |
| | MOX | 8–10 | KIII ANN | Fu et al. (2012) |
| | QMB | 8 | PCA | García-Martínez et al. (2011) |
| | EP | 12 | PCA | Guadarrama et al. (2001) |
| Threshold detection of aromatic compounds | MOX | 16 | PCA, PNN | Santos et al. (2010) |
| | MOX | 16 | PCA, PNN | Arroyo et al. (2009) |

(*Continued*)

**TABLE 14.1** Electronic Noses and Their Wine Applications (*cont.*)

| Application | Sensor Technology[a] | Number of Sensors | Data Processing Algorithm | References |
|---|---|---|---|---|
| Geographical classification | MS | 1 | PCA, SLDA, PLS-DA | Cynkar et al. (2010) |
| | MOX (Fox 3000) | 12 | LDA | Berna et al. (2009) |
| | SAW (Znose) | 1 | LDA | Duarte-Mermoud and Beltran (2009) |
| | MOX (PEN2) | 10 | PCA, LDA | Buratti et al. (2004) |
| | MOX | 14 | | Rodriguez-Mendez et al. (2004) |
| Wine evolution monitoring | MOX | 16 | PCA, PNN | Lozano et al. (2014) |
| | CP (Aromascan) | 32 | PCA | Pinheiro et al. (2002) |
| Grape variety classification | MOX | 16 | PCA, PNN | Aleixandre et al. (2009) |
| | MOX | 16 | PCA, PNN | Lozano et al. (2007a) |
| | MOX | 16 | PCA, PNN | Aleixandre et al. (2008) |
| | MOX | 16 | PCA, PNN | Lozano et al. (2007b) |
| | MOX | 14 | PCA | Villanueva et al. (2006) |
| | MS | 1 | PCA, PLS, LDA | Cozzolino et al. (2005) |
| Aroma prediction, correlation | MS | 1 | PCA, PLS | Cozzolino et al. (2008) |
| | MOX | 16 | PNN | Lozano et al. (2007a) |
| | MOX | 16 | PNN | Aguilera et al. (2012) |
| | MOX | 16 | PNN | Lozano et al. (2006) |
| | MOX | 16 | PNN | Lozano et al. (2005) |
| | MOX | 12 | GA | Buratti et al. (2007) |

[a] *Commercial models in brackets.*

namely, dimethylamine (DMA), trimethylamine (TMA), ethyl octanoate, 1-hexanol, ethanethiol, and ethyl acetate.

Arroyo et al. (2009) have assessed the quality of wine using an e-nose comprising of 16 tin oxide thin film based sensors. The same e-nose has been used to detect the main aromas in red and white wines (Lozano et al., 2006). In a study by Lozano et al. (2005), 29 typical aromas in white wine grouped in different families (floral, fruity, microbiological, herbaceous, and chemical) were recognized with an e-nose. Fig. 14.3 shows an example the PCA score plot of fruity aromas. Some papers also address the discrimination among different brands or types of wine (Lozano et al., 2006; Penza and Cassano, 2004).

## 14.3.2 Off-Odors and Frauds Detection

Quality of a wine can be affected by positive and negative quality factors, such as off-odors. Quick and accurate identification of off-odors are advantageous to both winemakers and wine merchants. For the winemaker, early remedial action often can correct the situation before the fault becomes serious or irreversible. Some of the most important off-odors producing compounds in wine are acetic acid (Macías et al., 2013; Lozano et al., 2011), ethyl acetate, cork-related and sulfur compounds (Santos et al., 2010). E-nose techniques have also been used to detect defects or spoilage, for example, caused by high concentrations of 4-ethylphenol and 4-ethylguaiacol (Berna et al., 2008; Cynkar et al., 2007); 2,4,6-trichloroanisole; and oct-1-en-3-ol (Martí et al., 2003; Ragazzo-Sanchez et al., 2009).

Another interesting application of e-nose technology is the detection of wine fraud in which there are attempts at misleading the wine drinker (and/or wine collector) into believing that he or she has bought a different/better product than is actually the case. Adulteration is the common word for many kinds of food and beverage fraud. Wine adulteration can be committed through dilution with water, addition of alcohol or other substances, and blending with, or replacement by, wine of a lesser quality. E-nose technology offers the possibility of realizing in situ analysis of wines and has been shown able to detect adulteration of wine with ethanol, methanol, and other substances (Penza and Cassano, 2004). Apart from such adulteration, wine fraud can be committed through misinformation about the wine, such as mislabeling related with the origin of the grapes and/or wine and the winemaking process. In this field, e-noses have been used to discriminate geographical origin (Cynkar et al., 2010; Berna et al., 2009; Duarte-Mermoud

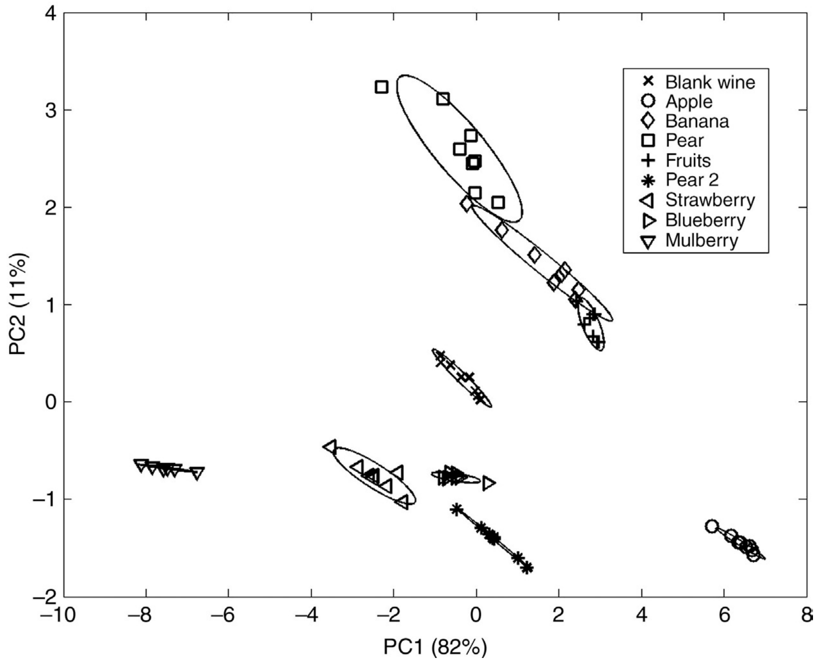

**FIGURE 14.3** **PCA plot of the measurements of fruity aromas in white wine.** *(With permission from Elsevier Science.)*

and Beltran, 2009; Buratti et al., 2004; Rodriguez-Mendez et al., 2004) and they can also be used to detect frauds in differentiating traditionally aged wines from artificially aged wines (Santos et al., 2011).

## 14.3.3 Grape Variety Classification and Special Grape Treatments

An important characteristic, which determines the flavor and character of a wine, and hence its quality, is the grape variety from which it is produced. Since the grape variety is responsible for different aromas in wines, the discrimination of the variety of grapes used in the wine elaboration can be achieved with machine olfaction technology. A study by Aleixandre et al. (2009) presented the analysis and classification of different wines depending simultaneously on their denomination of the origin and the grape variety.

There have been many studies (Lozano et al., 2007a,b; Aleixandre et al., 2008; Villanueva et al., 2006; Cozzolino et al., 2005) that showed different experiments of e-nose prototypes used for grape variety classification (Airén, Chardonnay, Macabeo, Malvar, Pardina, Riesling, and Grenache, Mencía, Prieto Picudo, Syrah, Tempranillo, Tinta del País, Tinta de Toro).

Special grape treatments refer to applications of e-noses to differentiate volatiles of grapes and wines treated with an aqueous ethanol spray (Zoecklein et al., 2011; Martin et al., 2008), dealcoholization of samples (Ragazzo-Sanchez et al., 2005), the effect of grapevine canopy side (Devarajan et al., 2011), and the effects of sun-drying and dehydration (Lopez de Lerma et al., 2012).

## 14.3.4 Wine Elaboration Process Monitoring and Wine Aging

The wine elaboration process, including wine aging in oak barrels, is critical for obtaining quality wines. Oak barrels are commonly used in the aging of wine and spirits because of the barrel's positive effects in their sensory characteristics. The identification of wine elaboration (Lozano et al., 2014) and aging process has a great importance for origin denominations for control of frauds. As an example, an e-nose was used for recognition and detection of wine aging (Lozano et al., 2008a): the same wine was aged in different types of oak barrel (French and American oak) and during different lengths of time (0, 3, 6, and 12 months); Fig. 14.4 shows the results in a PCA plot. Another application is the discrimination of wine samples according to aging type: in an oak barrel or in stainless steel tanks with the addition of small oak wood pieces (Prieto et al., 2012; Santos et al., 2011).

## 14.3.5 Comparison with Other Techniques

Comparative studies among the responses from electronic nose and traditional wine analysis techniques dates back to the early 1990s.There has been a twofold motivation for developing such correlations: mainly with human panels and gas-chromatography and mass-spectrometry analysis. The quantifying method must perform some kind of regression analysis to establish a predictive model from the feature vector coming from the gas sensor responses to another set of continuous dependent variables, such as gas concentration.

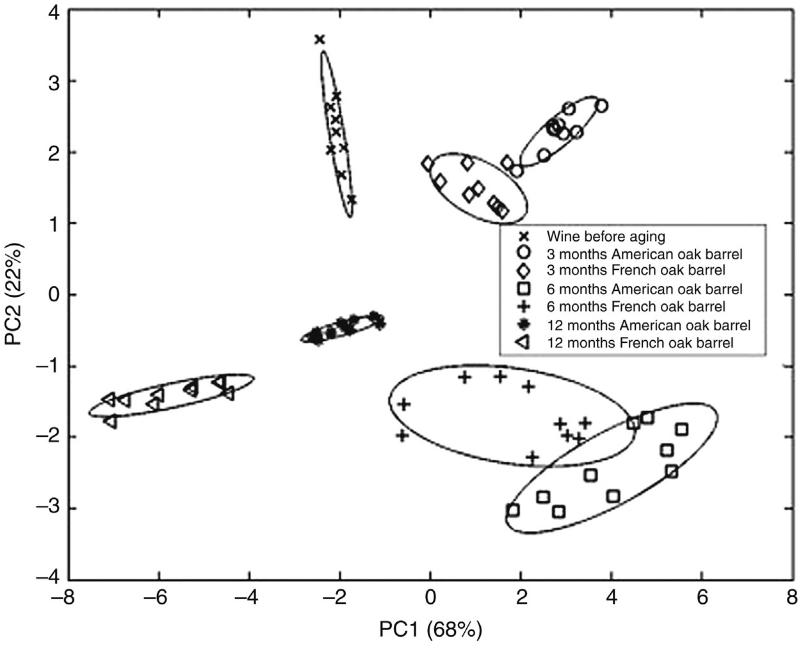

**FIGURE 14.4** **PCA score plot of measurements of wine samples aged in French and American oak barrels for 0, 3, 6, and 12 months.** *(With permission from Elsevier Science.)*

The regression can be of several types. One of the more common situations is the monitoring of a process variable (eg, quality level, gas concentration …) associated with an analyte into a mixture of unknown compounds or to make a sensory analysis in which the dependent variable is the response of a human sensory panel to the same analytes (eg, intensity, hedonic tone …). PCR and PLS are some of the regression methods that can be used to solve those problems.

The works that are most prevalent in sensor comparison with panels are related to wine quality testing. This is rather expected due to the rich and varied qualifiers often used in the wine industry. Arroyo et al. investigated a homemade e-nose and 25 human tasters trained to classify 17 different aromas, which are used to discriminate the quality of wine. It was found that human tasters performed better in identifying certain aromas (Arroyo et al., 2009). However, an e-nose subsequently developed by Santos et al. (2010) was found to be better in detecting the specific thresholds of typical red wine compounds such as ethyl acetate and eugenol and white wine compounds such as hexanol and ethyl octanoate. In another study, the success rate of an e-nose was compared to a sensory panel and a GC–MS through 28 wine samples. All the sensor signals were referenced to the signal from a mixture of ethanol (12% v/v and distilled water) for drift reduction and compensating ethanol effects. It was found that the results provided by the e-nose employing PLS regression algorithm corresponded better to the sensory panel results than to the predictions of GC–MS (Lozano et al., 2007a). Fig. 14.5 shows the estimated values of sensory panel attributes versus real values by PLS.

## 14.4 CONCLUSIONS AND FUTURE TRENDS

This chapter has attempted to provide information about recent advancements in the e-nose applications in the wine field, according to the four basic blocks of these devices: sampling systems, gas sensors, data acquisition, and signal processing. The main problems encountered in wine applications and the main features and differences with general-purpose e-noses have also been revised. The main applications found in the literature have also been reviewed (Table 14.1).

The concept "e-nose" is very attractive since researchers have attempted to mimick the mammalian sense of olfaction without its subjective component. In any case, e-noses are not commonly used in wine industrial processes. Some reasons could be related with the traditional and familiar sense of the wine industry. However, most of the well-known problems (robustness, selectivity, and reproducibility) of e-noses applied to wine must be solved.

First, there is no an ideal sampling method. The choice of the procedure depends on the objective of the study, as well as the matrix and the sensitivity of the analytical method. On the detector's side, major focus must be given to the design and development of drift-free sensors that can be used reliably over long temporal horizons, which could be a convincing factor for the wine industry when considering using such a device. Consequently, the internal drift influencing factors like crystalline structure variation, grain size variations, grain boundary effects, uniformity in the dopant concentrations, perfect contact materials, and thickness of

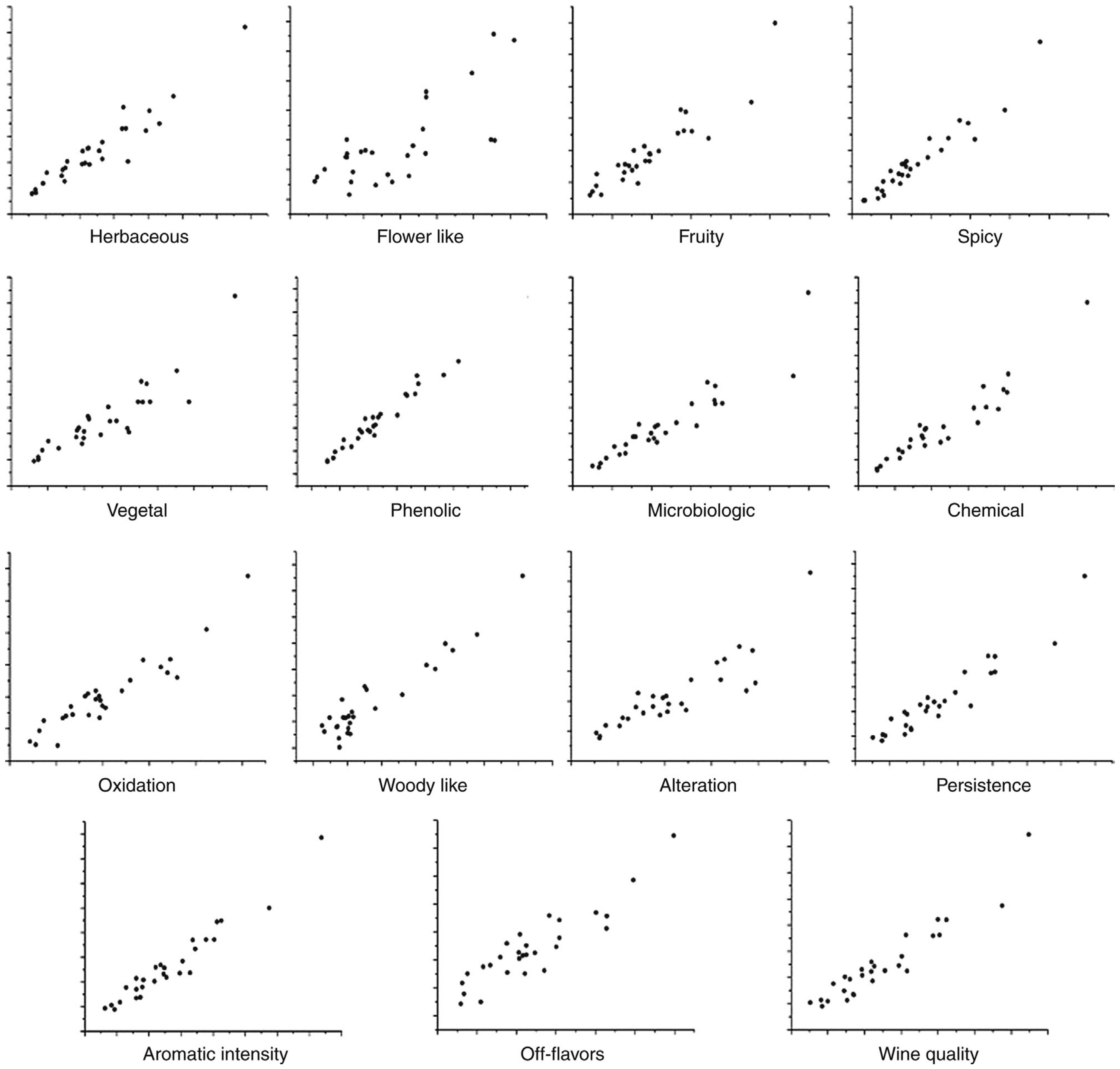

**FIGURE 14.5 Sensory panel attributes estimated by PLS model ($y$) versus real values ($x$).** *(With permission from Elsevier Science.)*

the sensing elements should be addressed. Nanotechnology will decisively influence the miniaturization and cheapening of e-noses, integrating gas sensors in smart devices, and creating large sensor networks.

Second, advanced instrumentation and electronics combined with advanced and miniaturized sensors could be applied to new fields in the entire wine production chain: from the vineyard (small sensor motes forming part of self-organizing sensor networks for control and monitor environmental parameters) to the end consumer (eg, in the corks to report the status of the wine) through the stages of transport and distribution. The integration of gas sensors in smart devices, RFID (radio frequency identification) tags, and other devices will contribute, as other technologies have some years ago, to widely extend artificial olfaction to millions of users.

Third, the signal and data processing is the last component of an e-nose technology. While the basic methodology to build qualitative or quantitative prediction models is firmly established, there are still limited literature regarding the use of signal and data processing to improve the robustness of the systems. The need to compensate for sensor drift, humidity, and the selectivity requiring that an e-nose is trained to recognize specific patterns representing odors is a very important issue in artificial olfaction. Another problem of the advance in this field is that the developed techniques published are not available to the scientific community for testing and improving them. In a similar line of thought, data sets are not available like those that have occurred in other related fields, in order to compare the performance of the algorithm developed. We hope, in a near future, to see free codes and data sets properly documented, available for the scientific community.

Novel and amazing applications of e-noses are expected in the field of wine in the coming years that benefit both winemakers and users. It will be necessary to develop robust systems for long time measurements that can be easily maintained, and do not require expert technicians for handle. This is the main challenge for the e-nose community.

## ABBREVIATIONS

| | |
|---|---|
| **ANN** | Artificial neural network |
| **ANOVA** | Analysis of variance |
| **BPANN** | Back propagation artificial neural network |
| **CA** | Correlation analysis |
| **CDA** | Canonical discriminant analysis |
| **CP** | Conducting polymers |
| **CART** | Classification and regression trees |
| **CLA** | Cluster analysis |
| **DFA** | Discriminant factorial analysis |
| **EP** | Polymer-based electrochemical sensors |
| **FL** | Fuzzy logic |
| **GA** | Genetic algorithms |
| **ICA** | Independent component analysis |
| **LR** | Linear regression |
| **LSD** | Least significant difference |
| **MOX** | Metal oxide semiconductor sensor |
| **PCA** | Principal component analysis |
| **PCR** | Principal component regression |
| **PLS** | Partial least squares |
| **PNN** | Probabilistic neural networks |
| **RBFNN** | Radial basis function neural networks |
| **RSR** | Quadratic response surface regression |
| **SLDA** | Stepwise discriminant analysis |
| **SVM** | Support vector machine |
| **VOCs** | Volatile organic compounds |

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## Chapter 15

# Electronic Tongue Principles and Applications in the Food Industry

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## 15.1 INTRODUCTION

Food distribution and its safety are very actual problems of the modern society. While in advanced nations overconsumption is continuously growing, and it is especially pronounced in the food industry sector, in the other part of the globe, the famine is still a horrifying reality even nowadays. The security in food sector is required to ensure the availability of food, uniform distribution, quality, and safety. The latter depend on product chemical composition, physical properties, the level of microbiological and toxic contamination, and storage conditions; these are regulated by ISO 22000 standards (www.iso.org) and Hazard Analysis and Critical Control Points (HACCP) (http://www.fda.gov/Food/GuidanceRegulation/HACCP/).

Nowadays consumers have become more and more selective and demanding in regards of their diet. The taste and the quality of food are the primary tools for the consumer to express his or her preferences. The increased consumer requirements of food quality and safety issues have resulted to the development of new techniques for food authentication. However, most of these techniques are time consuming and require sophisticated apparatuses and skilled personnel (Pico, 2012). Due to these limitations, alternative analytical methods should be provided. A brilliant example of such an alternative approach for food assessment is an application of electronic tongue multisensory systems mimicking the human gustatory system functioning. The e-tongue is capable of determining food quantitative composition and recognizing (identifying, classifying, discriminating) different food tastes. Moreover, the artificial sensorial assessment of analyzed food products can be easily correlated to human perception. The modern electronic tongues permit to perform a fast and nondestructive evaluation of food quality both in the laboratory environment, and in online analyses during the industrial food manufacturing process. Several comprehensive reviews on e-tongue applications for foodstuff analysis have been previously published (Sĺiwińska et al., 2014; Escuder-Gilabert and Peris, 2010). There are several commercially available systems on the market, such as, α-Astree from AlphaMOS (France), the Insent® electronic taste sensing system from Anritsu Corp. (Japan), multiarray chemical sensor from McScience (Korea), and the E-tongue from Sensor Systems (St. Petersburg, Russia). These artificial taste systems, as well as home-made devices developed by different research groups, were intensively utilized and continue to meet a growing interest for various food assessment tasks.

In this chapter the principles and implementation of artificial taste systems for foodstuff analysis performed in the last 5 years are reviewed. The main attention is given to the analysis of solid foodstuffs, and such important fluids as milk and edible vegetable oils, whereas the beverages' assessment will be discussed in other chapters of this book.

## 15.2 ELECTRONIC TONGUE DEFINITION AND PRINCIPLES

According to the IUPAC definition, an electronic tongue is "a multisensor system, which consists of a number of low-selective sensors and uses advanced mathematical procedures for signal processing based on Pattern Recognition and/or Multivariate data analysis …" (Vlasov et al., 2005). Among the sensor types employed in the modern electronic tongue systems are electrochemical such as voltammetric (Men et al., 2013, 2014; Apetrei et al., 2013; Wei and Wang, 2011a,b, 2013; Rodríguez-Méndez et al., 2009; Ruiz-Rico et al., 2013; Campos et al., 2010, 2013; Medina-Plaza et al., 2014), potentiometric (Tortora et al., 2009; Dias et al., 2014; Hruskar et al., 2010a,b; Lvova et al., 2012; Gil et al., 2011; Major et al., 2011; Zakaria et al., 2011; Escriche et al., 2012; Sousa et al., 2014; Kutyła-Olesiuk et al., 2013a), impedimetric, or capacitive sensors (Angkawisittpan and Manasri, 2012; Ulloa et al., 2013) sensors, optical sensors (Tortora et al., 2009), enzyme-based biosensors

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(Medina-Plaza et al., 2014), and hybrid or hyphenated devices (Tortora et al., 2009; Apetrei et al., 2010; Haddi et al., 2013; Bougrini et al., 2014; Zakaria et al., 2011; Kutyła-Olesiuk et al., 2013b).

The concept of the e-tongue has been advanced in recent years and along with nonspecific chemical sensors implementation in the arrays of specific ion-selective electrodes (Lvova et al., 2012; Zakaria et al., 2011; Kutyła-Olesiuk et al., 2013a,b), and the application of novel future extraction techniques (Wei and Wang, 2011b; Ulloa et al., 2013) were reported. The global information obtained from the e-tongue is still utilized to obtain a digital fingerprint of product gustatory characteristics, but also concrete parameters and the contents of specific compounds can be determined by the appropriate choice of sensors and chemometric techniques. A measurement performed by artificial sensing systems generates a vast volume of data; these data are then used to treat with chemometric methods. The most often applied techniques are artificial neural networks (ANN), principal component analysis (PCA), cluster and hierarchical cluster analysis (CA, HCA), support vector machine (SVM), various regression methods: partial least squares (PLS), multiple linear regression (MLR), and principal component regression (PCR). The detailed description of these chemometric methods can be found elsewhere (Sĺiwinĺka et al., 2014).

The various sensing materials are employed in e-tongue technology, among them are metallic sensors (Wei and Wang, 2011a,b; Bougrini et al., 2014; Ruiz-Rico et al., 2013; Escriche et al., 2012; Campos et al., 2013; Tian et al., 2013), carbon-paste (Apetrei and Apetrei, 2014; Apetrei et al., 2010; Bougrini et al., 2014; Rodríguez-Méndez et al., 2009), chalcogenide glass electrodes (Zakaria et al., 2011), polymeric films (Toyota et al., 2011a,b; Yasuura et al., 2014; Ciosek et al., 2015; Jaĺczyk et al., 2010; Tortora et al., 2009; Lvova et al., 2012; Kutyła-Olesiuk et al., 2013a), molecular imprinted polymers (Bueno et al., 2014), and multitransduction coatings (Tortora et al., 2009). In the next sections, some selected applications of e-tongue technology based on these materials in food industry are presented.

## 15.3 SAMPLE PRETREATMENT REQUIREMENTS FOR FOOD ANALYSIS BY E-TONGUE

While previously electronic tongue devices were mainly used to analyze liquids, recently there have been much research on the assessment of solid, oily, fibrous, or nonaqueous foodstuffs. In this relation, e-tongue analysis meets several application problems concerned with the sample state and particular pretreatment procedure requirements. Thus, the solid food samples should be transformed into the appropriate phase state in order to be properly measured (and physically enter in close contact with sensitive materials). For instance, solid foods should be crushed or minced (Campos et al., 2010, 2013; Tian et al., 2013; Kutyła-Olesiuk et al., 2013b); the cold samples must be heated to the sensor operation temperature, the hot ones—cooled, nonuniform—homogenized by stirring of sonication (Rodríguez-Méndez et al., 2009; Ulloa et al., 2013), and so forth. Moreover, considering that most types of sensors applied in the modern e-tongue systems operate mainly in a liquid phase (and even more often, in aqueous media), the sample wetting, dilution, and/or extraction with "sensor-friendly" solvents are required (Men et al., 2013; Dias et al., 2014; Apetrei and Apetrei, 2013; Haddi et al., 2013; Wei and Wang, 2011b, 2014; Ulloa et al., 2013). These processes are similar to the preprocessing that the human mouth applies to a food while eating: the mastication to diminish the food pieces size, the insalivation to start initial food transformation with saliva enzymes that prepare it for the further passage to the digestion tract, and the tasting that occurs on the particular tongue zones, sensitive to the five basic tastes.

## 15.4 TASTE AND TASTE COMPOUNDS DISCRIMINATION BY E-TONGUE

The flavor assessment and the taste-determining compounds detection were and still remain one of the most common applications of e-tongue systems since the very first reports dated in the early 1990s (Hayashi et al., 1990). The five basic tastes influencing the overall flavor of food are sweet, salty, bitter, sour, and umami (or delicious). Recently a kokumi term has been introduced to describe product "complexity," "mouthfulness," and "long-lastingness." The molecules responsible for basic tastes are sugars bearing carbonyl groups for sweetness, mainly sodium ions for saltiness, hydrogen ions for sourness, and monosodium glutamate that is often introduced in foodstuffs to enhance the "appetitive" umami taste. A plenty of compounds and among them many natural toxins have bitter taste; that is why, in order to provide natural protection reaction, the human tongue is extremely sensitive to bitterness. One of the reference bitter-tasting compounds is quinine. The most potent *kokumi* substance known to date is a peptide molecule composed of glutamine, valine, and glycine amino acids.

In the past, the attempts to correlate the basic taste compounds content to food flavor were not in general successful, since the taste sensation is a complex process determined by the combination of several chemical, physicochemical, and biological parameters of food (Rosenthal, 1999). Nevertheless, the basic taste compounds are often used to calibrate artificial sensing systems for qualitative monogustatory characterization tasks. Thus, Toyota et al. (2011a,b) have studied in details the sweetness responsible compounds detection in food, and developed the sweetness sensors to be applied in the taste sensing system for nonelectrolytes (sugars and sugar alcohols) and positively charged artificial sweetener compounds, like aspartame (Yasuura et al., 2014). An application of interdigital capacitor e-tongue for determination of sugar content in sugar solutions was reported by Angkawisittpan and Manasri (2012). Other applications are related to the bitterness intensity evaluations of drags (Liu et al., 2014a) and dairy protein hydrolysates (Newman et al., 2014), an assessment and comparison of umami taste coming from different flavor enhancers (Yang et al., 2013), discrimination of pharmaceutical formulations, and evaluation of the masking effect of active ingredient (normally bitter) in drugs by the addition of sweeteners and flavors (Ciosek et al., 2015; Guhmann et al., 2012; Jańczyk et al., 2010; Eckert et al., 2014).

## 15.5 APPLICATION AREAS IN FOOD

### 15.5.1 Olive and Vegetable Oils

Being one of the most consumed foodstuffs, vegetable oils, and in particular olive oils, have been actively assessed by multisensory systems over the last two decades, and still now the interest to vegetable oils analysis by artificial sensing systems is high. Traditionally the artificial olfaction systems, electronic noses, were mainly applied for the vegetable oils aroma assessment (Pico, 2012). The e-tongues' use was much less due to the experimental problems related to the obligatory sample pretreatment: the oils' should be extracted or diluted with hydrophilic solvents in order to permit a proper functioning of electrochemical sensors utilized in e-tongue. Due to the large variety of brands and trademarks of vegetable oils in the market, the main applications of electronic tongue systems for oils analysis are: the identification of the oil plant source material (Men et al., 2013; Tortora et al., 2009), the brand and/or geographical origin uniformity control (Haddi et al., 2013; Dias et al., 2014), the insurance of a product quality without adulterations (Tortora et al., 2009; Apetrei and Apetrei, 2014; Men et al., 2014), the identification of different oil components and their content quantification (Tortora et al., 2009; Rodriguez-Mendez et al., 2008), and the taste assessment (Dias et al., 2014; Apetrei et al., 2010).

Thus, Men et al. (2013) have reported a voltammetric e-tongue based on singular Au working electrode for classification of different types of edible oil coming, respectively, from corn, sunflower, soybeans, sesame, and peanuts. The oil samples were extracted with deionized water at 30°C for 5 min, and the aqueous phase was then analyzed with e-tongue. The system could discriminate all oils; the best identification result was obtained with PCA. Lately the same e-tongue device in combination with e-nose comprising commercial TGS Figaro gas sensors was applied to detect the blending ratio of the old frying oil and the new edible oil in the process of fried food manufacturing (Men et al., 2014).

The Portuguese and Spanish oils obtained from single olive cultivars were analyzed by means of a potentiometric e-tongue system comprising 40 screen-printed potentiometric sensors modified with cross-sensitive PVC membranes doped with different preestablished mass combinations of four lipidic additives by Dias et al. (2014). Polar compounds from each oil were extracted using a hydroethanolic solution ($H_2O$:EtOH, 80:20 v/v). Oils were correctly classified according to olive cultivar and e-tongue sensitivity was greater than 97%. Moreover, the system ability to sense polar compounds present in olive oils gave a possibility to assess indirectly the organoleptic properties like bitterness, astringency, or pungency.

An electronic tongue system based on square wave voltammetry and carbon paste sensors modified with edible oils was applied by Apetrei and Apetrei (2014) for the detection of adulterations of an extra virgin olive oil with different percentages of sunflower oil, soybean oil, and corn oil. A possibility to classify correctly the adulterated oils when the concentration level of adulterant oil was between 5 and 10% and to evaluate polyphenol's content was shown. Previously the same authors (Apetrei and Apetrei, 2013) have reported the detection of phenolic content of extra virgin olive oils by array of polypyrrole modified screen printed electrodes.

In our previous work, the vegetable oils of different plant sources (seed and olive) were tested by opto-potentiometric e-tongue, based on porphyrin-doped multitransduction sensors to detect possible defects or falsifications (Tortora et al., 2009). The oils have been extracted by methyl and ethyl alcohols (500 μL of oil with 3 mL of alcohol). Oil extract (100 μL) was injected in 0.01 M background KCl carrier solution flowing with 1 mL/min rate through the transparent measurement cell. The same sensing film produced during the analysis the optical and potentiometric responses, and the data obtained from array were treated simultaneously and gave a clear oil's discrimination. Moreover, the application of multitransduction e-tongue has permitted the possibility to monitor the content of linolenic, linoleic, L-glutamic, and L-ascorbic acids in oils (Fig. 15.1).

The application of hyphenated artificial sensing systems simultaneously employing e-nose, e-tongue, and e-eye were reported recently for the characterization of olive oils with different degree of bitterness (Apetrei et al., 2010) and for improved classification of Moroccan virgin olive oils (Haddi et al., 2013). In particular, in Apetrei et al. (2010), the combination of e-nose based on metal oxide (MOX) gas sensors, voltammetric e-tongue with carbon paste electrodes modified with olive oils, and e-eye based on transmittance spectra, recorded using a series of LEDs in the range from 780 to 380 nm, gave a clear discrimination of 25 extra virgin olives in function of olive variety. A root mean square error

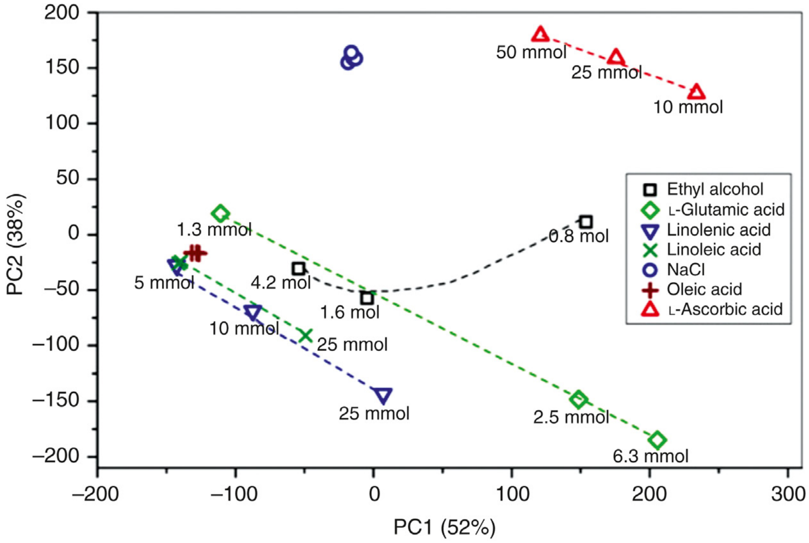

**FIGURE 15.1** **PCA score plot of combined opto-potentiometric ET system response toward several oil components.** *(Reprinted from Tortora et al., 2009, with permission from Elsevier.)*

of prediction (RMSEP) was lower than 0.099. The correlations among the electronic panel data and oil bitterness index (scored by a panel of experts), and the polyphenolic content (measured by chromatographic methods) were established by PLS method and the correlation coefficients higher than 0.9 were found both in calibration and validation. A low level of abstraction data fusion was applied in Haddi et al. (2013) to merge the data from e-nose composed from five commercial MOX sensors and voltammetric e-tongue based on platinum, gold, glassy carbon, and indium tin oxide (ITO) electrodes for correct identification of five Moroccan virgin olive oils according to their geographical origin. For electrochemical measurements, oils were dissolved in dichloromethane containing tetrabutyl-ammonium tetrafluoroborate as supporting electrolyte and analyzed at 30°C using a water bath. The capability of discrimination of hyphenated system was superior to the two instruments taken separately.

## 15.5.2 Milk and Dairy Products

The dairy industry produces a wide spectrum of products, comprising many types of milk, yogurts and other fermented drinks, soft and aged cheeses, and sour creams. Many fermentation techniques, microorganisms, and food additives are used in dairy industry, and several types of raw milk coming from different mammal animals are utilized. The necessity to maintain the product uniformity, to guarantee the freshness, and to avoid adulterations brings to the growing number of electronic tongue applications to dairy products analysis (Wei and Wang, 2011b; Wei et al., 2013a,b; Hruskar et al., 2010a,b; Bougrini et al., 2014; Lvova et al., 2012).

Thus, Wei and Wang (2011b) have developed a voltammetric e-tongue composed of five metallic electrodes to detect the residues of six different antibiotics in bovine milk. Antibiotics may pass into milk from animals that are routinely medicated to promote growth and for therapeutic and prophylactic reasons. The detection of antibiotics in raw milk is important, since they may negatively influence the further milk fermentation or even provoke allergies in consumers. Milk samples were spiked with antibiotic samples (1 mg was dissolved in 100 μL of DMSO and then diluted to 100 mL solutions with milk). The possibility was demonstrated to clearly distinguish bovine milk adulterated with different antibiotics by discriminant function analysis (DFA) method was demonstrated. The quantitative prediction of antibiotics concentration was performed by PLS with all the correlation coefficients $R^2$ over 0.9. Lately the same research group has reported a direct application of voltammetric e-tongue based on four metallic electrodes (Au, Ag, Pt, Pd) to monitor the quality and storage time of 26 samples of pasteurized milk at seven different times spread over 72 h after unsealing (Wei et al., 2013b) and for the evaluation of varieties of set yogurts and their physical properties (Wei et al., 2013a).

The comparison of two methods, sensory analysis and αAstree potentiometric e-tongue based on chemical field-effect transistors (chemFETs), for monitoring changes and classification of commercial brands of probiotic fermented milk of different flavors was performed by Hruskar et al. (2010a). The appearance, consistency, color, odor, and flavor evaluated by trained panelists were correlated with e-tongue response over the 20-day period of milk storage on two different temperatures (+4 and +25°C) by means of

ANN and PLS methods. The possibility of e-tongue to classify probiotic fermented milk according to flavor, to monitor degradation during storage, and to predict the sensory characteristics and their relationship to the milk quality was demonstrated. Another application of αAstree e-tongue was reported for simultaneous determination of ethanol, acetaldehyde, diacetyl, lactic acid, acetic acid, and citric acid content in 40 samples of probiotic fermented milk (Hruskar et al., 2010b). The data obtained from E-tongue were correlated by ANN to the amounts of milk components determined by enzymatic method through reaction with corresponding enzymes.

A combination of voltammetric e-tongue consisted of four metallic working electrodes (Pt, Au, Ag, and glassy carbon) and of a hybrid e-nose (composed from home-made and commercial MOX sensors, temperature probe, and humidity sensor) was applied to differentiate pasteurized milk brands and to recognize their storage time by Bougrini et al. (2014). Before performing measurements, the milk samples were allowed to reach ambient temperature and analyzed without any other pretreatment by e-tongue and e-nose. Treated separately, the e-tongue data have permitted a clear distinction of the milk brands on the first storage day, leading to 80.8% of the total variance. The combination of the e-nose and e-tongue data (midlevel abstraction fusion was applied) yielded PCA classification of all the milk storage days (Fig. 15.2). The SVM applied to fused data provided a complete identification of pasteurized milk storage days.

Previously we reported an application of multisensory systems for "in vitro" monitoring of salt release from model domestic soft cheeses during digestion in artificial gut system and for discrimination among commercial Italian mozzarella cheeses produced from bovine and buffalo milk on the base of their salinity (Lvova et al., 2012). The amount of $Na^+$ was evaluated by high-performance liquid chromatography (HPLC) technique and compared to the data from two ion-selective electrodes (ISE) sensitive to sodium (Na-ISE 1, from Metrohm IonAnalysis, and home-made Na-ISE 2 based on monensin dodecyl ester) and ISE array comprising five home-made ISEs for chloride, potassium, ammonia, calcium, and nitrate. It was found the better performance of ISE array for $Na^+$ content determination in comparison to singular selective sensors. A PLS result for $Na^+$ predicted with ISE array had a correlation coefficient 0.952, slope 0.887, and RMSEP 14.4 mM. Moreover, the salinity of commercial mozzarella cheese samples, and utilized milk type (bovine or cow), were satisfactorily determined with the ISE array. For this 2 g of fresh mozzarella cheese or of partially digested cheese sampled from an artificial mouth or different compartments of the artificial gut system were reduced in fragments (if needed) and extracted with 10 mL of distilled water. The solid part was centrifuged and 1 mL of liquid extract was collected and utilized for further sensory analysis. In total, 87.5% of mozzarella cheeses were correctly identified (Fig. 15.3).

## 15.5.3 Fish and Meat

The accurate quality and safety control of foodstuffs rich of proteins, providing nutritious health care compounds, meat and seafoods in particular, has received much attention due to the extreme importance of such products in a balanced diet. The freshness is the major issue in the fish and meat market industry, and multisensory analysis has been found an effective tool for shelf-life and postmortem time monitoring. Several works related to the e-tongue systems application for fish freshness control

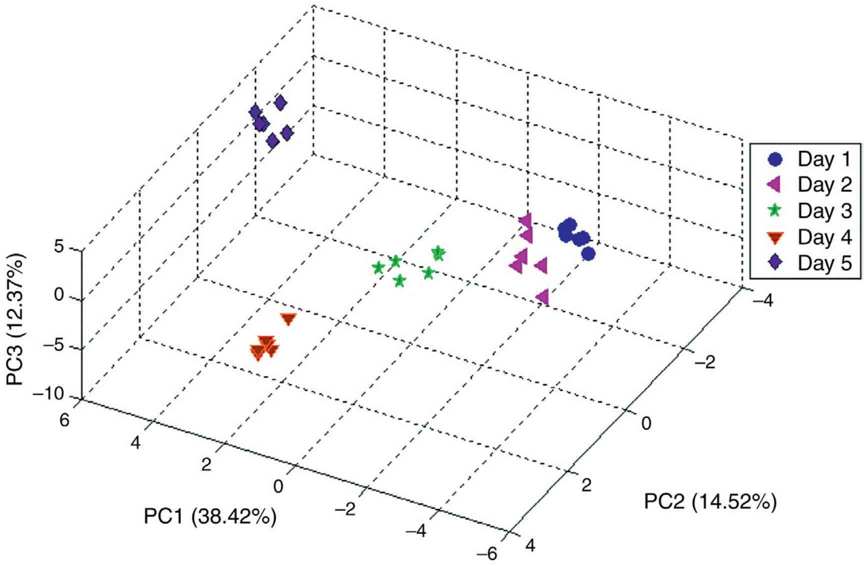

**FIGURE 15.2 PCA score plot for 5 storage days for "Jawda" pasteurized milk measurements gathered with the hybrid e-nose and the voltammetric e-tongue.** *(Reprinted from Bougrini et al., 2014, with permission from Elsevier.)*

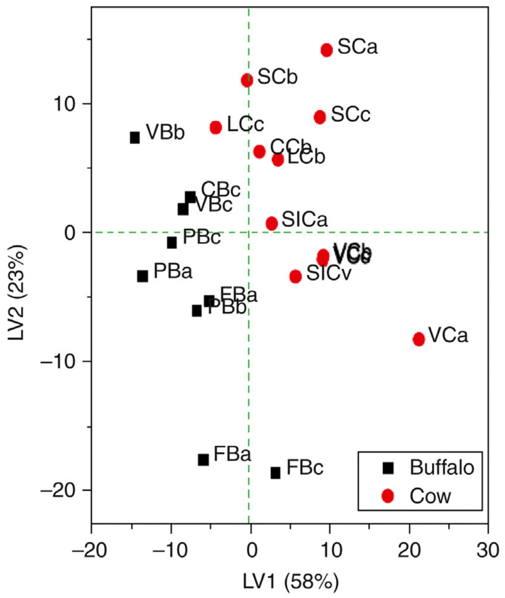

**FIGURE 15.3** **PLS-DA discrimination of 16 mozzarella cheeses made of buffalo and cow milk.** *(Reprinted from Lvova et al., 2012, with permission from Elsevier.)*

(Rodríguez-Méndez et al., 2009; Apetrei et al., 2013; Han et al., 2014), quality assessment (Ruiz-Rico et al., 2013), and taste (Liu et al., 2014b) were previously reported. Thus, Rodríguez-Méndez et al. (2009) have detected the different amines (ammonia, dimethylamine, trimethylamine, cadaverine, and histamine) during the Cyprinid family fish spoilage process by arrays of voltammetric carbon paste and screen-printed electrodes (SPEs), modified with phthalocyanines. The PCA identification and partial least squares discriminant analysis (PLS-DA) evaluation of fish freshness and determination the fish postmortem period was performed. Prior to measurement, 1 g of the fish muscle was cut, extracted by 5 min sonication in 25 mL of a 0.1 M KCl; the liquid phase was separated by filtration and used in e-tongue measurements. The same research group has demonstrated recently an utility of polypyrrole-modified SPEs in voltammetric e-tongue for the Pontic shad fish freshness monitoring (Apetrei et al., 2013).

An application of voltammetric e-tongue, including metallic electrodes grouped in two arrays, one made up of noble metals (Ir, Rh, Pt, Au) and another of nonnoble ones (Ag, Co, Cu, Ni) for shelf-life assessment of fresh cod fish was reported by Ruiz-Rico et al. (2013). Electronic tongue measurements were performed directly on each fish sample at room temperature (Fig. 15.4). The total volatile basic nitrogen (TVB-N), pH, moisture, ATP-related compounds, mesophilic bacteria, and Enterobacteriaceae counts were evaluated by standard techniques and correlated to e-tongue data. A successful PLS fitting was obtained for TVB-N and mesophilic bacteria, two of the main fish spoilage indices thus confirming the potential usefulness of the voltammetric tongue for assessing cod spoilage.

In plenty of tasks, electronic tongues are often used for classification applications rather than evaluation of taste. For taste assessment, an interesting research aimed to isolate and study the structural properties of flavor peptides from raw, cultured puffer fish muscle was reported by Liu et al. (2014b). Different fractions of fish muscle were isolated and the flavor peptides contained in these fractions were assigned the possibility to elicit different tastes (umami, bitter, kokumi, etc.) according to the peptide structures, which were identified by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI–TOF–MS) method.

Gil et al. (2011) have applied an array composed by Au, Ag, Cu, Zn, Pb, and graphite potentiometric electrodes for the monitoring of the pork loin freshness over a 10-day period storage under refrigeration. Data gathering in meat was carried out by sticking the set of electrodes and the reference electrode directly on the meat slice sample. At every measurement the new meat piece was analyzed. Data were taken for no less than 5 min in order to allow the electrodes to reach the equilibrium. A remarkable correlation was observed between e-tongue data and *K-index* (measures the variation in the adenosine triphosphate, ATP, degradation products) through PLS regression. Additionally, PCA and ANN analyses showed that is was possible to determine the meat postmortem time. The same research team has reported the application of e-tongue based on pulse voltammetry and composed of a set of noble (Au, Pt, Rh, Ir, Ag) and nonnoble (Ni, Co, Cu) electrodes for detection of chloride, nitrite and nitrate concentrations in brines and in minced meat (Campos et al., 2010).

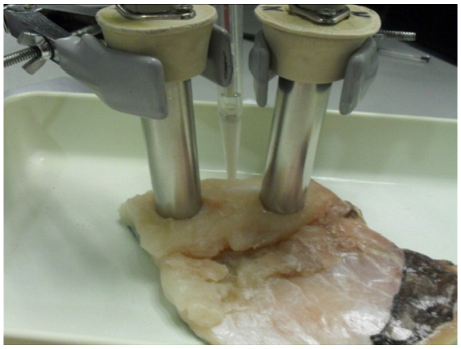

**FIGURE 15.4** **Electronic tongue measurement on the cod samples.** *(Reprinted from Ruiz-Rico et al., 2013, with permission from Elsevier.)*

## 15.5.4 Honey

The nutritional and therapeutic values of honey make it one of the most appreciable natural products. The characterization of commercial honey is required to satisfy the consumers' demands. Being a viscous mixture of hundreds of components, honey meets several difficulties during its characterization, related to the necessity of samples' pretreatment and components separation, which are rather complex and expensive. As an alternative to standard instrumental methods, several applications of electronic tongue for honey's analysis were previously reported (Wei and Wang, 2011a, 2014; Major et al., 2011; Zakaria et al., 2011; Ulloa et al., 2013; Escriche et al., 2012; Sousa et al., 2014). The main focus of these works was on honey's botanical origin tracing and geographical classification. The common pretreatment procedure for honey prior to e-tongue analysis is a dissolution in water (hot or room temperature) and further measurements of obtained samples. For instance, Wei and Wang (2011a) reported a possibility to discriminate deferent monofloral honey by metallic voltammetric e-tongue and PCA according to their floral origins. More recently the same authors have compared the ability of previously mentioned voltammetric e-tongue to the performance of α-Astree potentiometric e-tongue to trace both floral and geographical origins of monofloral honey (Wei and Wang, 2014). Both devices were correctly able to forecast honey's categories by PLS, PCR, and least squared-support vector machine (LS-SVM) methods, and the regression models for predicting the four types of honey of different geographical origins by the e-tongue were very stable.

Major et al. (2011) have demonstrated the utility of α-Astree e-tongue for botanical classification of 12 samples of acacia, chestnut, and honeydew honey by PCA, ANN, and canonical correlation analysis (CCA) modeling; additionally the physicochemical parameters of honey such as electrical conductivity (0.999), acidity (0.997), water content (0.994), invert sugar content (0.988), and total sugar content (0.979) were evaluated by ANN correlation of e-tongue data to the reference values determined by traditional methods.

Zakaria et al. (2011) have performed the discrimination of honey coming from different botanical origins and monitored their adulterations with sugar by separated and fused measurements with Cyranose 320 (Smith Detection$^{TM}$) e-nose and chalcogenide-based potentiometric e-tongue made up of seven distinct ion-selective sensors from Sensor Systems (St. Petersburg, Russia). The highest classification score (94.44%) was observed by probabilistic neural network (PNN) when applying sensor fusion. Ulloa et al. (2013) have reported a successful discrimination of four commercial brands of Portuguese honey according to their botanical origin by fusion of impedance electronic tongue and UV–Vis–NIR spectroscopy assisted by PCA and CA chemometric techniques. The e-tongue consisted of four working electrode plates of aluminum, gold, platinum, and ITO. The capacitance ($C$, nF), conductance ($G$, μS), and conductance/angular frequency ratio data were collected from e-tongue; data were preprocessed to find response curve fitting coefficient features that were then applied for the honey's classification together with spectroscopy fitted data and analyzed with multiway PCA (MPCA) yielding 100% classification. It was shown that fused data was better to determine the floral origin of honey varieties than when using e-tongue and especially spectroscopic data separately.

Escriche et al. (2012) made an attempt to classify the honey samples in three different states according to the applied thermal treatments (raw, liquefied, and pasteurized) by means of potentiometric electronic tongue based on various metals (Au, Ag, and Cu) and metallic compounds ($Ag_2O$, AgCl, $Ag_2CO_3$, and $Cu_2O$) electrodes. No satisfactory discrimination was found; instead, the PCA and ANN analysis showed that e-tongue is useful to classify honey by its botanical origin. A remarkable PLS correlation between the e-tongue response and honey's physicochemical parameters such as color by Pfund scale ($R^2 = 0.958$), luminosity ($R^2 = 0.935$), and diastase activity ($R^2 = 0.926$) were found. Another potentiometric e-tongue based on 20 cross-selective lipid membranes was applied for classification of

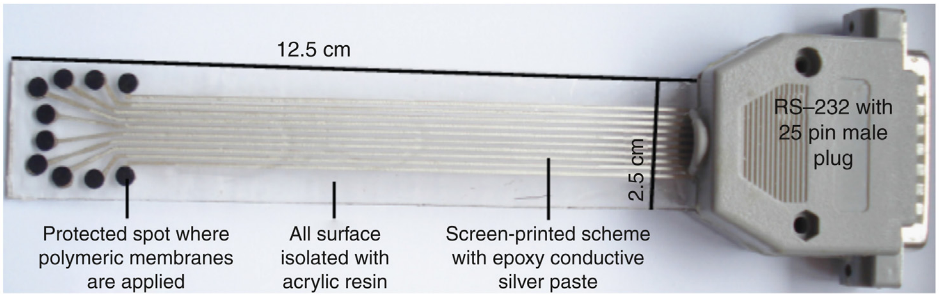
12.5 cm
RS–232 with 25 pin male plug
2.5 cm
Protected spot where polymeric membranes are applied
All surface isolated with acrylic resin
Screen-printed scheme with epoxy conductive silver paste

**FIGURE 15.5** **Multisensor system for honey discrimination.** *(Reprinted from Sousa et al., 2014, with permission from Elsevier.)*

65 monofloral Portuguese honey (Sousa et al., 2014). The multisensor system was printed in both sides of a PVC board using a print-screen technique (Fig. 15.5). It was demonstrated that after a preliminary selection of honey according their colors (white, amber, and dark) e-tongue was able to correctly classify honey with a high variability in floral origin.

### 15.5.5 Fruits and Vegetables

An assessment of fruits and vegetables' freshness, sweetness, and quantitative evaluation of several nutrient components, antioxidants, and vitamins' content is another area of multisensory systems employment. Among different fruits, grapes are especially important since they are starting product of wine. The grapes analysis is often focused to evaluation of phenolic antioxidants. Thus, Medina-Plaza et al. (2014) have reported a e-tongue system formed by nanostructured voltammetric biosensors based on phenol oxidases (tyrosinase and laccase) for the discrimination of grapes of different varieties according to their phenolic content. The sensors were prepared by incorporation of enzymes into Langmuir–Blodgett (LB) film of arachidic acid (AA) doped with lutetium bisphthalocyanine ($LuPc_2$) as electron mediator. The grape samples were tested in form of musts diluted 50% in water. The PCA scores plot has demonstrated that bioelectronic tongue is able to discriminate phenols according to the number of phenolic groups attached to the structure and satisfactory discriminate among five grape varieties (Fig. 15.6).

Campos et al. (2013) reported the use of a metallic voltammetric e-tongue to monitor the ripeness of seven Spanish grape varieties. The grapes were crushed after collection and the juice separated from the pulp was analyzed. A good PLS predictive ability of e-tongue for total acidity and sugar content with errors under 15% was reported.

The group of Wang employed α-Astree potentiometric e-tongue and voltammetric metallic e-tongue for the discrimination of preserved licorice apricots (Tian et al., 2013) and evaluation of firmness and sugar content in pears of different cultivars (Wei and Wang, 2013). The apricots were softened in deionized water, minced, and the supernatant was measured by E-tongue after centrifugation. For peer analysis the juices squeezed from the middle part of a fruit and filtered using double-decked filter papers were tested.

Kutyła-Olesiuk et al. (2013a) have used a flow-through analytical system based on miniaturized ISE array to evaluate the effect of lead accumulation in maize leaves. The

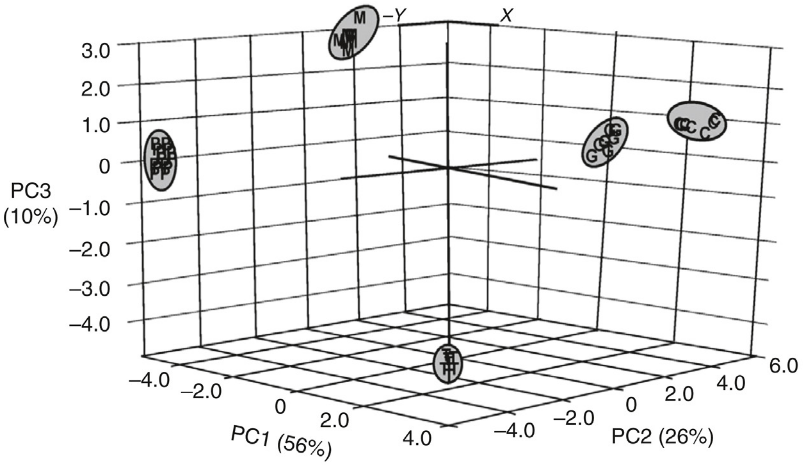

**FIGURE 15.6** **PCA scores plot of the different varieties of grapes.** *T*, Tempranillo; *G*, Garnacha; *C*, Cabernet; *M*, Mencia; and *PP*, Prieto Picudo. *(Reprinted from Medina-Plaza et al., 2014, with permission from Elsevier.)*

leaves harvested from 3 to 4-week-old plants were exposed to 5–10 mM lead nitrate solutions for 24 h and then lyophilized. For potentiometric measurements, the plant material (0.2 g of dry weight) was mixed with 0.3 mL of 96% $H_2SO_4$ and 10 mL of 30% hydrogen peroxide and then tested in flow-through mode. It was demonstrated that the developed e-tongue system with PLS-DA could be a potential tool for the estimation of the cultivation conditions of plants during bioindication or phytoremediation. The same researchers employed a hybrid electronic tongue in the qualitative and quantitative analysis of aqueous extracts obtained from raw and dried apples prepared by different drying techniques (Kutyła-Olesiuk et al., 2013b). The system included five potentiometric ISEs, Au and glucose oxidase (GOx) based amperometric sensors, spectrophotometric and conductometric measurements. It was shown that the combination of the data from various measurement techniques leads to improved differentiation of the dried apple extract samples compared to separate methods.

### 15.5.6 Other Food-Related E-Tongue Applications

Other recent applications of e-tongue related to the food analysis deal with specific protein profiling (Bueno et al., 2014), bacteria and/or food pathogens detection (Escriche et al., 2012; Poshtiban et al., 2013), allergens screening (Peres et al., 2011), and pet food control (Eves et al., 2013).

## 15.6 CONCLUSIONS

An application of electronic tongue systems based on chemical sensors or hyphenated techniques for food assessment represents the evident advantage of ease of use and low costs. The speed analyses, minimal sample preparation required, the possibility of the automation, and fast, effective screening of large numbers of formulations in a short period of time make e-tongue a promising substitute technology to sensory analysis performed by testers. Moreover, the electronic tongue approach represents a fast and nondestructive way to evaluate food quality not only in the laboratory environment, but also during the online food manufacturing process and may provide additional quantitative information on specific food constituents.

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## Chapter 16

# Beer Analysis Using an Electronic Tongue

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## 16.1 INTRODUCTION

The sense of humans is obscure and subjective. Let us suppose the situation where a boy, his father, and his grandmother drink green tea. The boy says "this green tea is bitter," whereas his father says "it is mild," but his grandmother says "this tea is not bitter." These different responses occur due to the difference of the acceptable range (capacity) of bitterness of each person. In other words, the criterion of each person's taste is based on a different scale. Furthermore, the taste is determined by a number of factors, including the five senses (sight, hearing, touch, taste, and odor) as well as food habit and dietary culture.

Sensory evaluation has been made to estimate the tastes of samples so far. This method has several problems such as low objectivity, low reproducibility, the stress possibly imposed on panelists, and the significant cost of selecting and training panelists. It is difficult to carry out sensory evaluations because of the potential for medication side effects in the medical and pharmaceutical field. On the other hand, quantitative analysis using gas and liquid chromatography cannot be used to estimate the intensity of each basic taste. Therefore, objective methods of evaluating tastes without using the aforementioned human sensory systems have attracted attention. The development of objective methods of evaluating taste contributes greatly to the better qualities of foods and beverages and the compliance of drug products.

In this chapter, the principle of the taste sensor, that is, electronic tongue with global selectivity, and its application to beer measurement are explained in detail.

## 16.2 TASTE SENSOR—ELECTRONIC TONGUE WITH GLOBAL SELECTIVITY

A taste sensor comprises several kinds of electrodes, on which a lipid/polymer membrane is pasted, and can discriminate, identify, and quantify the taste of foods or drug products; that is, it provides a "scale of taste" (Habara and Toko, 2006; Kobayashi et al., 2010; Tahara and Toko, 2013; Toko, 1996, 2000a,b, 2013; Toko et al., 2013). It is now commercialized as Taste Sensing Systems SA402B and TS-5000Z and used throughout the world; TS-5000Z and its sensor electrodes are shown in Fig. 16.1a,b, respectively. The taste sensor utilizing lipid/polymer membranes is a kind of electronic tongue. It was developed in 1989 by the patent application and introduced in an academic paper in 1990 (Hayashi et al., 1990). It has been applied to many kinds of foods such as beer (Ikezaki et al., 1991; Tahara and Toko, 2013; Toko, 2000b; Toko et al., 1994), coffee (Fukunaga et al., 1996; Ishiwaki, 2013), sake (Arikawa et al., 1996; Iiyama et al., 1996), milk (Mizota et al., 2009; Yamada et al., 1997), green tea (Hayashi et al., 2010, 2008; Ikezaki et al., 1997), black tea (Hayashi et al., 2013; Uchiyama et al., 2011), wine (Baldacci et al., 1998; Totsuka, 2013), soy sauce (Iiyama et al., 2000), miso (soybean paste) (Imamura et al., 1996), salts (Chen et al., 2010), meat (Chikuni et al., 2010; Sasaki et al., 2005), and rice (Tran et al., 2004, 2005), and has also been used to measure the taste of amino acids (Akitomi et al., 2013; Miyanaga et al., 2004; Toko and Fukusaka 1997) and medicines (Harada et al., 2010; Takagi et al., 2001, 1998; Uchida et al., 2001, 2013).

The taste sensor has a new concept of global selectivity, which means the decomposition of a chemical substance into taste qualities and their quantification, rather than the discrimination of individual chemical substances (Anand et al., 2007; Habara and Toko, 2006; Kobayashi et al., 2010; Tahara and Toko, 2013; Toko, 1996, 2000a,b, 2013; Toko et al., 2013; Riul et al., 2007). As a result, the taste sensor responds consistently to the same taste, similarly to the human tongue. The property that each sensor electrode is not specific to each chemical substance is one of the noticeable properties of electronic tongues (Anand et al., 2007; Citterio and Suzuki, 2008; del Valle, 2010; Escuder-Gilabert and Peris, 2010; Ghasemi-Varnamkhasti et al., 2010; Latha and Lakshmi, 2012; Riul et al., 2007; Savage, 2012; Winquist, 2008; Woertz et al., 2011a,b), which is similar to biological receptors in the gustatory system. The features

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of electronic tongues for measuring liquid using multiple sensor arrays to measure liquid are (1) low selectivity and high cross-selectivity instead of high selectivity and (2) the capability of statistically analyzing the outputs from multiple sensors. These features (ie, low selectivity, high cross-selectivity and statistical analysis) are similar to those of electronic noses for measuring gas.

The human taste receptors do not necessarily recognize individual chemical substances. Each of the receptors for the five basic taste qualities (saltiness, sweetness, bitterness, sourness, and umami) simultaneously receives multiple chemical substances. It means the human gustatory receptors have a semiselective property or global selectivity. In chemical analysis methods such as liquid and gas chromatography, therefore, it is practically impossible to measure the taste of foods containing several hundred types of taste substances. In addition, there are interactions between different tastes and between taste substances; for example, the bitterness of coffee is suppressed by adding sugar and a synergistic effect for umami can be obtained by mixing two kinds of umami substances represented by amino acids and nucleotide-derived substances.

The taste sensor is an electronic tongue with global selectivity as previously mentioned. It is composed of several kinds of sensor electrodes, and hence a multicomponent analysis is sometimes utilized in a similar way; however, each electrode of the taste sensor is specific to each taste in principle.

Let us briefly review the history of development and improvement of taste sensor about this point. The first model of taste sensing system SA401 was put on the market in 1993 following the research results in 1990 (Hayashi et al., 1990) obtained by Toko's group. The taste sensor at this stage was capable of the classification of various foods and beverages. However, there were some problems to overcome on the taste sensor membranes. Each of the membranes had low selectivity to similar taste substances, and hence all the sensor outputs had be analyzed together by such a multivariate analysis as the principal component analysis (PCA). PCA is one of the powerful mathematical techniques used to obtain useful information by reducing a large number of variables to as few alternative variables as possible without losing information. However, the data resulting from PCA cannot be interpreted uniquely as taste information unless information on taste qualities for all samples is given before the analysis. In other words, discrimination and identification of foods and beverages are possible using multivariate analyses, but taste evaluation for the development of food and beverage products cannot be made.

To overcome this problem, each lipid/polymer membrane was required to respond more selectively to chemical substances with a similar taste and to recognize different taste qualities by itself. Lipid/polymer membranes were improved drastically in order to achieve these points (Kobayashi et al., 2010). As a result, each membrane can respond to a similar taste in a similar way, but can respond to a different taste in a different way. Each independent sensor electrode is now developed for measuring each taste quality (sourness, saltiness, bitterness, umami, sweetness, astringency). Several kinds of membranes have been developed for measuring sweetness and bitterness because there are a large number of compounds with various chemical structures and sizes in sweet and bitter substances. Three kinds of membranes have been fabricated specifically for sweet substances because there are three types of substances with different chemical structures and sizes as represented by sugars (glucose, sucrose), positively charged high-potency sweetener (aspartame), and negatively charged high-potency sweetener (saccharine sodium, acesulfame potassium) (Toyota et al., 2011; Yasuura et al., 2014a,b).

Four kinds of bitterness sensors have been developed for measuring acidic bitter materials, bitter hydrochloride salts, and basic bitter materials from the same reason (Kobayashi et al., 2010; Tahara and Toko, 2013; Toko, 2013; Toko et al., 2013). The bitterness sensor C00, as shown in detail later, has high sensitivity and selectivity to, for example, iso-α-acid, which produces a bitter taste in beer. This sensor membrane includes a positively charged lipid that can interact selectively with negatively charged bitter substances, which is also called acidic bitter substances. On the other hand, the bitterness sensors BT0, AC0, and AN0 contain a negatively charged lipid to interact with basic bitter substances that are positively charged. The bitterness sensor BT0 has been developed to highly and selectively respond to such bitter substances utilized mainly in the medical field (Kobayashi et al., 2010; Tahara and Toko, 2013; Toko, 2013; Toko et al., 2013).

Among the improvements of taste sensors, an invention of the CPA (change of membrane potential caused by adsorption of chemical substances) measurement is notable (Habara and Toko, 2006; Ikezaki et al., 1997; Kobayashi et al., 2010; Tahara and Toko, 2013; Toko, 2013; Toko et al., 2013). The CPA measurement enables us to estimate the aftertaste felt by humans. The term "CPA" originates from "change in membrane potential caused by adsorption of taste substances onto the membrane." The CPA measurement has a large merit to selectively measure the taste due to adsorptive substances such as bitterness and umami (Habara and Toko, 2006; Hara et al., 2014; Ikezaki et al., 1997; Kobayashi et al., 2010; Tahara and Toko, 2013; Toko, 2013; Toko et al., 2013, 2014). The amount of adsorption is affected by two factors, that is, the surface charge density and the hydrophobicity of the membrane. The CPA value is also affected by two factors, that is, the surface charge density of the membrane and the amount of adsorption (Toko et al., 2014).

The taste sensor is required to detect the interaction between taste substances or taste qualities; one of the typical interactions is suppression of bitterness due to sweet substances or bitter-masking agents. The suppression was detected successfully (Takagi et al., 1998, 2001) and this result is utilized for development of medicines (Harada et al., 2010; Toko, 2013). A familiar phenomenon we experience is a mellow effect of fat. The foodstuffs industry uses many edible oils in order to improve flavor and making the foodstuffs more palatable. The taste sensor clarified that bitterness and astringency is decreased by addition of edible oil to taste solutions, and that oil selectively suppresses bitterness and astringency, making foods taste milder (Kobayashi et al., 2010).

## 16.3 SEVEN KINDS OF TASTE QUALITIES

We will briefly summarize here the physiological knowledge of taste. The taste perceived by us is composed of five kinds of basic taste qualities: sourness, saltiness, sweetness, bitterness, and umami (Kawamura and Kare, 1987; Pfaffmann, 1959). The fifth taste, "umami," is sometimes called savoriness by many people from its property (strictly speaking, it is not the same) and is acknowledged as an independent taste found by a Japanese scientist (Kawamura and Kare, 1987). Sweetness is produced by sugar, glucose, and artificial sweetener, and generally becomes our source of energy. Saltiness is produced by cations such as sodium ions; however, anions also contribute to saltiness because we perceive a similar saltiness, but a different taste, if anion species are changed.

Sourness, a taste typical to vinegar, is caused by hydrogen ions generated from citric acid, acetic acid, and hydrochloric acid, for example. It activates metabolism, but generally is a signal of rot.

Bitterness gives a warning of toxicity. There are many kinds of chemical substances tasting bitter, as represented by caffeine, theobromine, quinine, and isohumulon. The iso-α-acid contained in hops is usually a mixture of mainly *cis*- and *trans*-isohumulone. Alcohol drinks, represented by beer, contain several kinds of bitter substances as previously mentioned.

Umami is the taste produced typically by monosodium glutamate (MSG) contained in seaweeds. Other typical umami substances are disodium inosinate (IMP) mainly contained in fish and meat and disodium guanylate (GMP) contained in mushrooms. Umami plays the role of supplying indispensable amino acids and nucleotides to our bodies. There is a synergistic enhancing effect in the coexistence of MSG and IMP (or GMP), which is characteristic to umami.

The other two kinds of taste are pungency and astringency. Chemical compounds that taste pungent are received at sensory receptors that are sensitive to temperature and pain. They are not received at taste receptors in gustatory cells. The major pungent compounds are allyl isothiocyanate, capsaicin, and piperine. Astringency is caused by polyphenol, mainly tannin. These compounds are said to mainly stimulate pain receptors. In the physiological meaning, astringency is similar to bitterness; hence, astringency is not strictly distinguished from bitterness, and the total strength of these tastes is considered to be important in some coffee makers.

## 16.4 MATERIALS AND METHODS IN THE BEER MEASUREMENT

The taste sensor, that is, the taste sensing system shown in Fig. 16.1, consists of several (at most, eight) kinds of working electrode with a lipid/polymer membrane used to receive taste substances, a handle, and a data processing unit. The working electrode has a structure of an Ag/AgCl electrode and the inner solution (3.3 M KCl saturated AgCl) contained in a polyvinyl chloride hollow rod, with which a lipid/polymer membrane is attached. Measurement using the taste sensor is based on the potentiometric principle, and

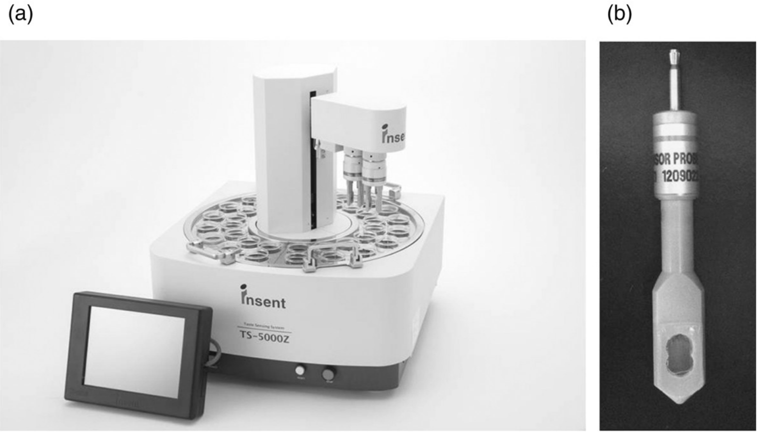

**FIGURE 16.1** (a) Taste sensing system TS-5000Z (Intelligent Sensor Technology, Inc.) and (b) the used electrodes.

**TABLE 16.1** Chemical Composition of Lipid/Polymer Membrane of Sensor Electrode for Each Taste Quality

| Taste Sensor Electrode | Taste | Lipid | Plasticizer |
|---|---|---|---|
| AAE | Umami sensor | Phosphoric acid di(2-ethylhexyl) ester, trioctylmetylammonium chloride | Dioctyl phenylphosphonate |
| CT0 | Saltiness sensor | Tetradodecylammonium bromide 1-hexadecanol | Dioctyl phenylphosphonate |
| CA0 | Sourness sensor | Phosphoric acid di(2-ethylhexyl) ester, oleic acid, trioctylmetylammonium chloride | Dioctyl phenylphosphonate |
| C00 | Bitterness sensor (for acidic bitter materials) | Tetradodecylammonium bromide | 2-Nitrophenyl octyl ether |
| AE1 | Astringency sensor | Tetradodecylammonium bromide | Dioctyl phenylphosphonate |

hence, the electric potentials between working electrodes for taste sensing and a reference electrode are measured. These electrodes use a silver wire coated with AgCl. The electric potential of the lipid/polymer membrane changes by both electrostatic interaction with taste substances and their physicochemical adsorption.

Table 16.1 summarizes five kinds of lipid/polymer membranes used in the sensor electrodes for beer analysis, in which the composition of lipids and plasticizers are shown. The composition of the lipid/polymer membrane is designed by considering the membrane electric charge density and the hydrophobicity on the basis of physicochemical properties of substances with each basic taste; the membrane electric potential changes when, for example, bitter substances are adsorbed onto the membrane owing to the electrostatic and hydrophobic interactions, whereas it changes when protons bind to the functional groups of lipid molecules appearing at the membrane surface in the case of sourness. As a result, a bitterness sensor, that is, a sensor electrode to measure bitterness, is relatively hydrophobic with a lower content of charged lipids. A saltiness sensor, that is, a sensor electrode to measure saltiness, is relatively hydrophilic with a higher content of charged lipids and can easily induce the electrostatic interaction with ions. This bitterness sensor that was so fabricated responds to acidic bitter substances contained in beer, green tea, black tea, Oolong tea, coffee, and wine (Kobayashi et al., 2010; Tahara and Toko, 2013; Toko, 2013). The detection limit for iso-α-acid is about 0.001 wt%, which is close to the human threshold. While the membranes for five taste qualities (sourness, saltiness, bitterness, umami, astringency) are shown in Table 16.1, the membranes for sweetness elicited by sugars or artificial sweeteners have been also developed (Toyota et al., 2011; Yasuura et al., 2014a,b).

The procedure for fabricating a taste sensor electrode is as follows:

1. The necessary types and amount of lipid(s) and plasticizer(s) are added to tetrahydrofuran (THF) and mixed for 1 h.
2. Polyvinyl chloride is added to this solution and then mixed for another 1 h.
3. The mixture is poured into a petri dish to dry it at room temperature for 3 days.
4. As result, a lipid/polymer membrane approximately 200 μm thick is created in the dish, and then is attached to the surface of a sensor probe using THF as an adhesive.
5. After 2 days of drying, the taste sensor electrode is complete.

This membrane fabricated as previously indicated can be used about 3000 times. The measurement is made as follows: first, the sensor electrodes are immersed in the first reference solution (30 mM KCl, 0.3 mM tartaric acid), and the membrane potential for the reference solution (reference potential), $V_r$, is measured. Next, the sensor electrodes are immersed in the sample solution ("beer" in the present case) for 30 s, and the membrane potential ($V_s$) is measured; the difference, $V_s - V_r$, is defined as the relative value, which corresponds to usual taste felt by humans. Then, the sensor electrodes are again immersed in the second reference solution (30 mM KCl, 0.3 mM tartaric acid) for 30 s, and the membrane potential for the second reference solution is measured again ($V_r$'), and the difference between $V_r$' and $V_r$, that is, $V_r' - V_r$, is defined as the CPA value, which corresponds to aftertaste experienced by humans. Finally, the membrane is rinsed with a sensor rinsing solution (100 mM KCl, 10 mM KOH, 30 vol% ethanol). This procedure is repeated five times for each sample, and the averages of the relative values and CPA values in the second measurement to the fifth measurement are used as the relative value and CPA value of each sample, respectively.

The CPA value is affected by two factors, that is, the surface charge density of the membrane and the amount of adsorption. It is shown that iso-α-acid molecules, typical to bitterness of beer, are adsorbed onto both the surface and interior of the lipid/polymer membrane of bitterness sensor, which has the low lipid content (Toko et al., 2014). The distribution coefficient (logD) of iso-α-acid is around 0.4

at weak acidic and neutral pH values. Therefore, it is very natural that iso-α-acid is adsorbed into the hydrophobic part of the lipid/polymer membrane of bitterness sensor, which was fabricated so that it might become hydrophobic, as previously mentioned.

Transformation of the sensor output to the sensory value felt by humans is very easy because the threshold (ie, sensitivity) of sensor output is near the human threshold for each taste quality, and furthermore the response increases with logarithm of the concentration of taste substances in a similar way to humans. The magnitude of sensor output is larger for taste substances for which humans feel stronger taste. These facts imply that the linear transformation enables us to get the taste information, that is, the sensory value of each taste.

As the Weber–Fechner law states, the relationship between a stimulus and the corresponding perceived intensity is logarithmic (Pfaffmann, 1959). In this context, the smallest detectable increase for the gustatory sense is about 20% (Schutz and Pilgrim, 1957). Therefore, we have defined "taste information" on the basis of sensor outputs and their characteristics. Let us consider the case of saltiness as an example. The saltiness sensor shows a slope 50 mV/decade with the NaCl concentration (Kobayashi et al., 2010). When the 1% NaCl concentration increases by 20%, it becomes 1.2%. Furthermore, this 1.2% concentration increases by 20%, and then it becomes 1.44%. This 20% increase in the concentration equals the smallest detectable increase for humans, and keeps constant on a logarithmic scale. Therefore, it is reasonable that the 20% increase is defined as 1 unit. This relationship leads to a slope 0.25 unit/mV; this conversion factor provides taste information for "saltiness" by its multiplication with the saltiness sensor output.

Each conversion factor for each taste sensor electrode can be thus calculated as the slope of a 10-fold concentration difference between the reference solution and a corresponding solution, which is one of the standard samples.

## 16.5 ANALYSIS OF BEER

Let us review briefly the results on beer reported previously using the taste sensor (Ikezaki et al., 1991; Toko, 2000b; Toko et al., 1994) before explaining the results obtained using the above mentioned method and sensor electrodes. Since the sensor membranes in this age (Ikezaki et al., 1991; Toko, 2000b; Toko et al., 1994) have no selectivity to individual taste quality, the response to beer is simply shown as an electric-potential pattern constructed from eight outputs of eight sensor electrodes. Fifteen brands of beer were measured (Ikezaki et al., 1991), and discrimination of beer was attempted. Three discrimination methods using squared Euclidean distance, standardized squared Euclidean distance, and Bayesian probability were adopted. As a result, the accuracy rate was 94.6, 100, and 100%, respectively. This good result is due to the fact that the standard deviation of sensor output is from 0.2 to 0.3 mV, whereas the difference between different brands of beer amounts to over 2 mV. In another paper (Toko et al., 1994), 36 brands of beer were measured. Discrimination of beer was easy from the same reasons as the previous study.

Expression of the taste such as rich, light, sharp, and mild is also possible by the comparison between the sensor outputs and the sensory tests by humans, as outlined by Toko (2000). The rich or light taste may arise from the concentrations of alcohol, hops, and so on. The PCA was applied to the output pattern. Comparison with human taste sense implied that PC1 corresponds to rich taste and light taste, and PC2 to sharp taste and mild taste. A high correlation of sensor output with the alcohol content of beer was obtained. A good linear relationship between the sensor output and human sensory evaluation (rich taste) was also confirmed. Furthermore, high correlations with pH and the bitter value estimated from iso-α-acid were obtained. These results first implied the usefulness of the taste sensor for quantifying human sensory expressions and physicochemical quantities in beer.

Let us return to the subject using a new set of sensor membranes listed in Table 16.1. Fig. 16.2 shows the transient responses of five kinds of sensor electrodes (AAE, CT0, CA0, C00, and AE1 in Table 16.1). We can see that the measurement is completed within 1 s, and that the slow changes occur with time. While this slow change is largest for astringency, it can be considered to occur with a process of the slow adsorption of astringent substances onto/into the membrane; in fact, the adsorption of astringent substances is confirmed, and a large amount of substances penetrates into the membrane (Hara et al., 2014).

Fig. 16.3 shows the taste pattern constructed from five axes of sourness (CA0), umami (AAE), bitterness (C00), aftertaste of bitterness (CPA of C00), and aftertaste of umami (CPA of AAE) for six brands of beer such as Budweiser, Sapporo Yebisu, Bass Pale Ale, Heineken, Kronenbourg1664, and Carlsberg. The taste was evaluated using the linear transformation of sensor output obtained from the measurement for 30 s as previously mentioned. The taste of Budweiser wass taken as the origin. The aftertaste of umami caused a sort of richness felt by humans.

We can see that six brands of beer each showed different taste patterns. The standard deviations of sourness, umami, bitterness, aftertaste of bitterness, and aftertaste of umami for Carlsberg were 0.05, 0.01, 0.11, 0.12, and 0.10, respectively, while those for Sapporo Yebisu were 0.02, 0.05, 0.02, 0.13, and 0.10, in that order. Discrimination of each beer was easy because of these small standard deviations. The properties of their tastes resulted as follows: Heineken and Kronenbourg1664 showed a similar taste pattern with strong sourness and relatively strong bitterness. Bass Pale Ale showed a taste pattern that was smaller but similar to the patterns for Heineken and Kronenbourg1664. Sapporo Yebisu had very strong bitterness and comparatively strong richness, and Carlsberg had relatively strong bitterness but

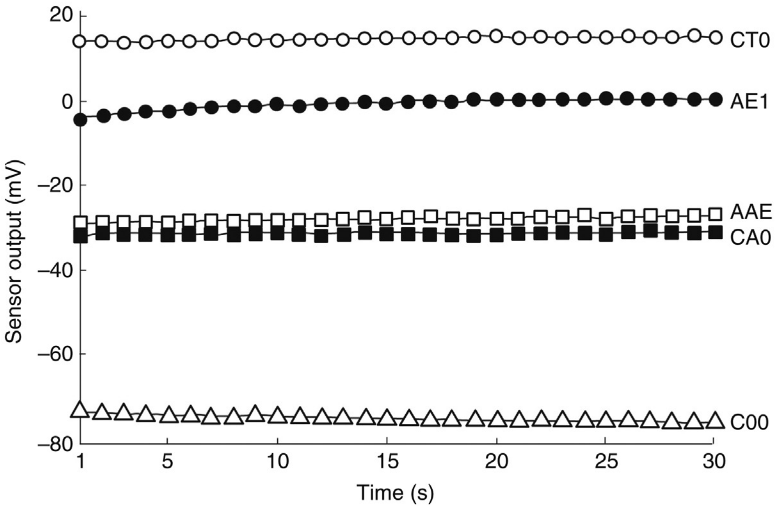

**FIGURE 16.2** Transient response of the sensor electrode to beer.

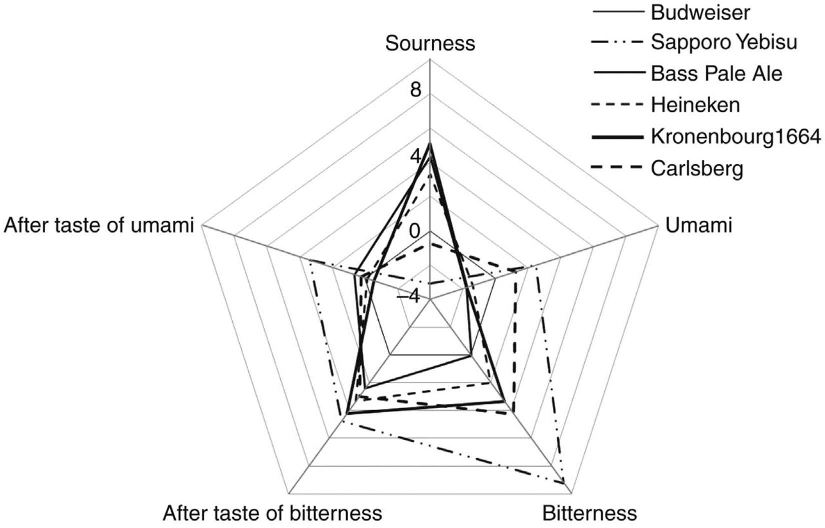

**FIGURE 16.3** Taste pattern for six brands of beer.

weak sourness. Budweiser showed fresh taste indicated by the weakest aftertaste of bitterness.

Fig. 16.4 shows a taste map of beer expressed by two axes of bitterness and sourness, obtained from the output of taste sensor. We can see the taste of beer in the world distributes in the wide range. Sapporo Yebisu, which is recognized as beer with strong bitterness from old days, tastes strongly bitter. The taste map shows that Budweiser tastes moderate, and that some brands of beer such as Kronenbourg1664, Bass Pale Ale, Castlemaine XXXX, and Moosehead Lager taste sour. Guinness and Mythos have relatively strong bitterness and sourness. The taste map provides the visualized information of taste as well as the discrimination of products. In this way, we can glance at the taste results received from the tongue using the taste sensor.

## 16.6 CONCLUSIONS

The taste sensor has resulted in a new concept of global selectivity. Discrimination of minute differences of molecular structures may not be important but the transformation of molecular information contained in interactions of substances with biological membranes into several kinds represented by taste intensities and qualities is important. The taste sensor makes this possible.

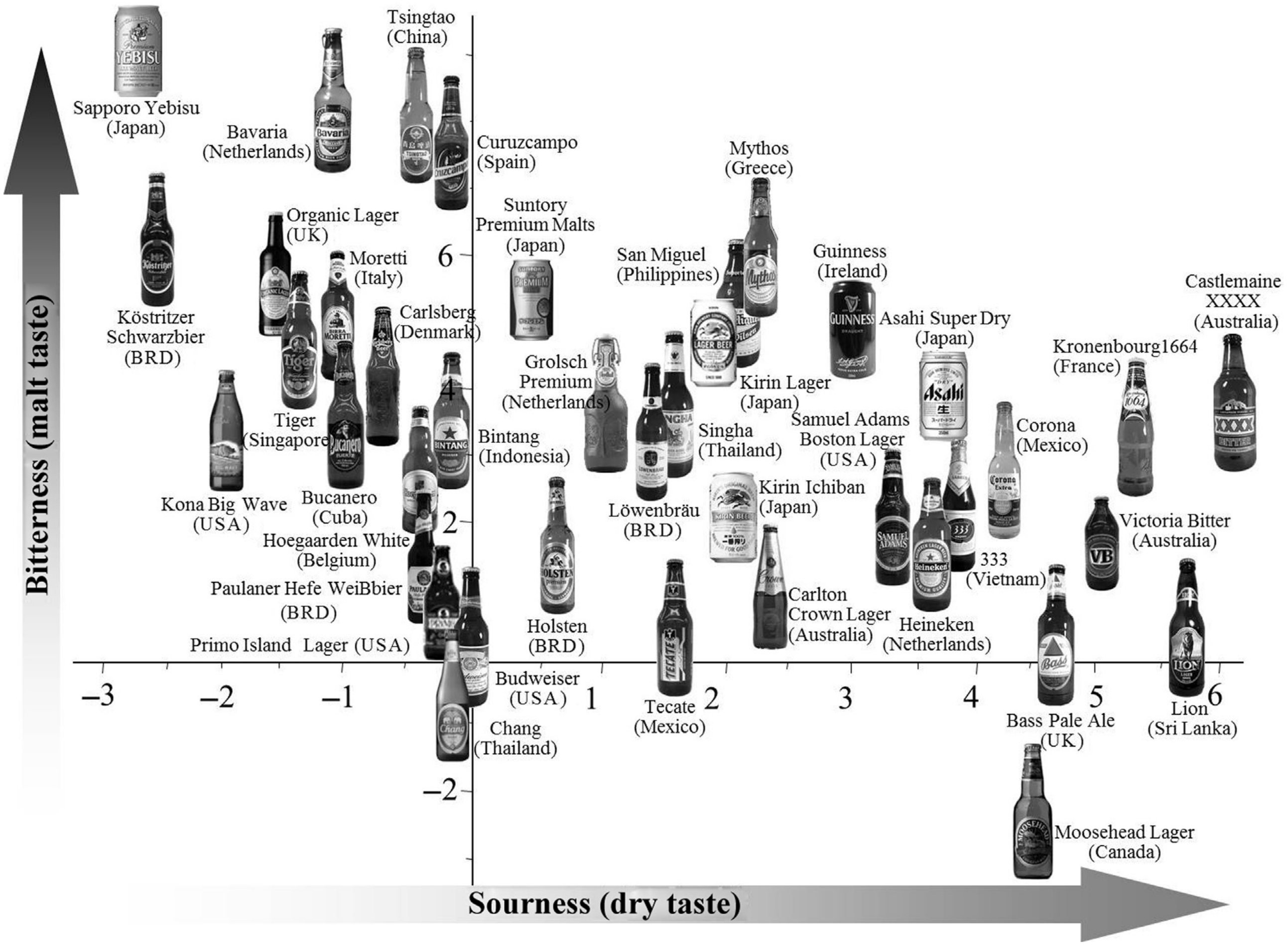

**FIGURE 16.4** Taste map of beer.

Taste of beer was quantified and at the same time the discrimination of beer was made using the taste sensor comprised of lipid/polymer membranes. Strict quality control is required in food and beverage industries. The taste sensor can play an important role in detecting deteriorated taste qualities, as reviewed (Habara and Toko, 2006; Kobayashi et al., 2010; Tahara and Toko, 2013; Toko, 2013; Toko et al., 2013). In addition to evaluating deterioration, the taste sensor can detect differences between product lots (Habara and Toko, 2006; Kobayashi et al., 2010; Tahara and Toko, 2013; Toko, 2013; Toko et al., 2013). A portable taste sensor has also been developed (Tahara et al., 2011, 2013), and will be applied for measurement of foods including beer anytime and everywhere. The taste sensor chip consists of a reference electrode and multiple working electrodes fabricated on polycarbonate substrates, polyimide double-faced tapes, and partitions. The taste sensor is one of the devices that provides an objective measure (scale of taste) to the ambiguous sense of taste. Taste becomes objective as well as other subjective quantities such as weight and length, which has already become objective by development of the corresponding method and device.

Therefore, the taste sensor can be used to provide taste information (kind and intensity of taste) to consumers and as an effective marketing tool in the food industry as well as to compare specific products with competitive products to determine the consumers' preference. At present, it is used not only for quality management but also for providing taste information for products as an added value. Moreover, the arbitrary tastes that we like can easily be created by utilizing the database obtained from measurements using the taste sensor. On the basis of this concept, coffee provided by Japan Airlines was designed using the taste sensor. As easily understood, manually making coffee with a desired taste would be a time-consuming trial-and-error task. However, the taste sensor enables us to accurately create a desired taste in a short period of time.

The taste sensor can be utilized to produce a new food or control the quality of foods as previously mentioned;

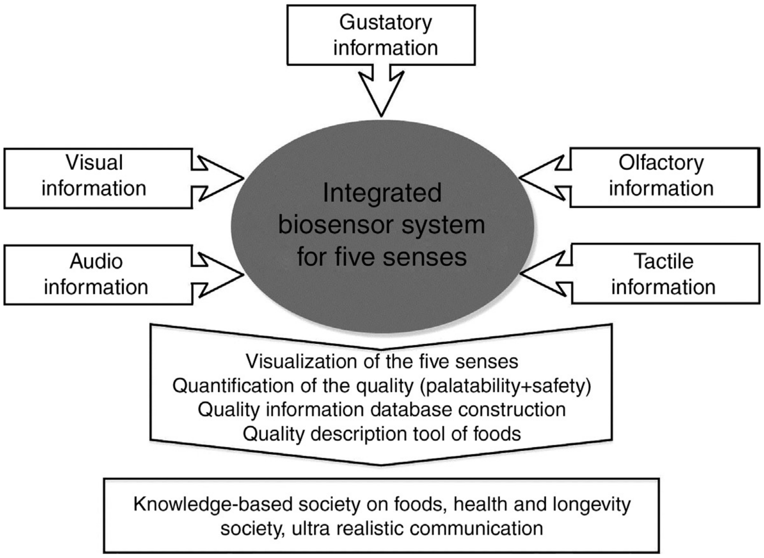
Gustatory information
Visual information
Audio information
Olfactory information
Tactile information
Integrated biosensor system for five senses
Visualization of the five senses
Quantification of the quality (palatability+safety)
Quality information database construction
Quality description tool of foods
Knowledge-based society on foods, health and longevity society, ultra realistic communication

**FIGURE 16.5** Integrated biosensor system to express palatability using sensor outputs corresponding to five senses.

furthermore, we can improve the taste easily using the taste sensor. Suppose that we measure a food that does not taste very good using the taste sensor. In this case, we can easily improve the taste on the basis of data in the taste sensor's archive. Of course, it is desirable for the palatability to be evaluated by also taking into account the history, food culture, and ethnicity concerned. Therefore, a mathematical formula to express the food score must contain these factors.

There are "musical scores" in our sense of hearing, and hence we can reproduce the music of, for example, Mozart or Beethoven today. If we can invent "food scores" in the sense of taste, in a way similar to musical scores, we should be able to preserve and transmit food scores, and then reproduce the taste of the desired foods anywhere and at any time. The spread of the Internet has enabled consumers to easily search for products that meet their requirements and expectations. This tendency will increase in the field of taste, and hence, the taste sensor will become more effective tools.

Humans perceive tastes by the tongue and also comprehensively sense tastes on the basis of information on the five senses, including odor, texture, visual appearance, and sound. Further study will develop an integrated biosensor system for the five senses to enable us to visualize the five senses, quantify the quality (palatability and safety), construct a quality information database, and develop a quality description tool of foods, as illustrated in Fig. 16.5. It will open a new world represented by a knowledge-based society on foods, a health and longevity society, and ultrarealistic communication.

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## Chapter 17

# Analysis of Coffees Using Electronic Tongues

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## 17.1 INTRODUCTION

Coffee is the most widely consumed beverage in the world, with an estimated 2 billion cups drunk daily (http://www.ico.org/about_coffee.asp). The effects of its regular consumption on health are of public interest, since coffee is a complex beverage containing several bioactive molecules that cause biological effects not limited to their biochemical actions. Caffeine is its most studied compound, but there are other compounds known to exhibit antioxidant properties and free radical scavenging activity, such as phenolic compounds (chlorogenic acids), diterpenes (cafestol and kahweol), and melanoidins (high molecular weight nitrogenous; Frost-Meyer and Logomarsino, 2012; Moreira et al., 2012; O'Keefe et al., 2013; Preedy, 2014). Various benefits may be obtained from regular consumption of coffee, for instance, in reducing the risk of infections, diabetes mellitus type 2, hypertension, and even obesity and depression (O'Keefe et al., 2013; Preedy, 2014). It may also act as an anticancer and neuroprotective agent (Preedy, 2014).

Some effects from regular consumption of coffee are attributed to specific substances. For instance, caffeine is believed to act as a stimulant to the nervous system and a cognitive performance improver, as well as a protective agent against dementia and Alzheimer's disease. Ingestion of 3–5 cups of coffee a day at midlife was associated with a reduction in the risk of dementia/Alzheimer's disease by ~65% at late life (Eskelinen and Kivipelto, 2010; Valls-Pedret et al., 2012). Caffeine can also be used to manage motor and nonmotor symptoms in Parkinson's disease (Prediger, 2010). Chlorogenic acid, another important substance in coffee, can present antiplatelet and antithrombotic effects besides hypocholesterolemic effect, which leads to atheroscleroprotective, cardioprotective, and hepatoprotective functions. Chlorogenic acid possesses a potent antihepatitis B virus activity and glucose regulation among other benefits (Wan et al., 2013; Fuentes et al., 2014; Preedy, 2014).

There are also problems for human health associated with coffee consumption. For instance, coffee can cause sleep disorders due to stimulant effects from caffeine. Other substances in coffee, such as cafestol and kahweol, can act as cholesterol raising, although possess a chemopreventative potential (Butt and Sultan, 2011; Singh et al., 2014). An association has been suggested between caffeine consumption (more than 151 mg/day) during pregnancy with both an increase in spontaneous abortion incidence and a decrease of infant weight at birth.

Overall, it seems that for adults the moderate consumption of 3–4 cups a day, that is, 300–400 mg of caffeine, there is little evidence of risks and some evidence of benefits for health (Preedy, 2014).

## 17.2 COFFEE ANALYSIS

The large consumption of coffee makes it one of the most important and valuable commodities, and therefore improving its taste is of great interest to the food industry (Preedy, 2014). Its characteristic flavor is derived from the grain, which is related to its varieties and influenced by agricultural treatment, drying procedures, fermentation, roasting, grinding, and packaging. Hence, an efficient quality control requires tools to monitor aspects that affect its flavor, in addition to considering new trends in consumption, for example, the use of coffee capsules, pods, and different machines (Várvölgyi et al., 2015). On the other hand, detection and quantification of specific substances, for example, adulterants and bioactive molecules such as caffeine, chlorogenic acid, and polyphenols, are becoming increasingly important.

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## 17.3 ELECTRONIC TONGUES USING ELECTROCHEMICAL TECHNIQUES

### 17.3.1 Coffee Discrimination

Pioneering papers on the analysis of coffee samples using electronic tongues (e-tongues) were published independently by two research groups in the 1990s. Fukunaga et al. (1996) published a paper entitled “Quantification of taste of coffee using sensor with global selectivity” in which a commercial taste-sensing system (SA401) of Anritsu Corp. was employed. The sensor array comprised seven electrodes made with lipid membranes as illustrated in Table 17.1. The principle of detection used was potentiometry, with the electrical potential being measured relative to the value obtained for coffee from Salvador (taken as the reference, ie, zero). The lipid membranes were made from polyvinyl chloride (PVC), the plasticizer dioctyl phenylphosphonate, and the lipid of interest, all mixed in a test tube in tetrahydrofuran. The mixtures were dried in a glass plate and kept at ca. 30°C. Finally, the lipid membranes were fitted to the seven hollow-cylinder electrodes.

The taste of 11 samples of coffee from different origins was analyzed, as follows: Salvador (CS), Brazil (Santos No. 2), Guatemala (SHB), Jamaica (Blue Mountain No. 2), Hawaii Kona (Extra Fancy), Kenya (AA), Tanzania (AA), Colombia (Excelso), Indonesia Mandheling (Grade 1), Indonesia (WIB 1), and Indonesia (AP 1). Upon applying the statistical tool of principal component analysis (PCA) to the results, it was possible to distribute the response electric potential patterns for 11 coffee samples on a two-dimensional plane, as shown in Fig. 17.1. The contribution rates of original data to PC1 and PC2 were 82.2 and 13.8%, respectively. This means that coffee taste can be quantified by at least two independent parameters, proven to be related to bitterness and acidity. Indeed, authors correlated the response electric potential of the taste sensor with data from a panel of nine human experts, who classified the coffee samples according to their acidity and bitterness. The correlation coefficients were 0.98 for acidity using channel 2 and 0.94 for bitterness using channel 5.

**TABLE 17.1** Lipids Used in the Electrode Membranes

| Channel | Lipid |
|---|---|
| 1 | Dioctyl phosphate (DOP) |
| 2 | DOP:TOMA = 9:1 |
| 3 | DOP:TOMA = 5:5 |
| 4 | DOP:TOMA = 3:7 |
| 5 | Trioctyl methyl ammonium chloride (TOMA) |
| 6 | Oleic acid |
| 7 | Oleyl amine |

Electrodes in channels 2–4 were hybrid membranes mixed according to the molar ratio specified in the table.

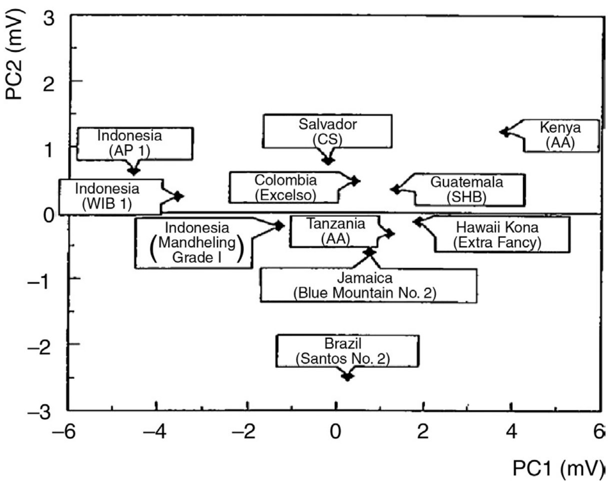

**FIGURE 17.1** PCA plot obtained with the results from the response electric potential for 11 samples of coffee from different origins.

Legin et al. (1997) reported on “Tasting of beverages using an electronic tongue” in which five types of coffee were analyzed using an e-tongue made with 21 potentiometric sensors. Details on the preparation of the sensing units can be found in the paper by Vlasov and Bychkov (1987). The samples were natural coffee (Tchibo, Manhattan, and Arabica) and instant coffee (Nescafe). PCA was also used to visualize the data generated by the 21-sensor array in a two-dimensional space. The results in Fig. 17.2 demonstrate good separation for all coffee samples, with natural coffees being distinguished from the instant coffees. Furthermore, distinction could be made of Nescafe coffee prepared using different technologies: “freeze dry” for Gold and “spray dry” for Classic.

In the work by Várvölgyi et al. (2015), a commercial device named Alpha Astree e-tongue (Alpha MOS, Toulouse, France) was used to distinguish between pure origin and blended coffee samples. This e-tongue comprised

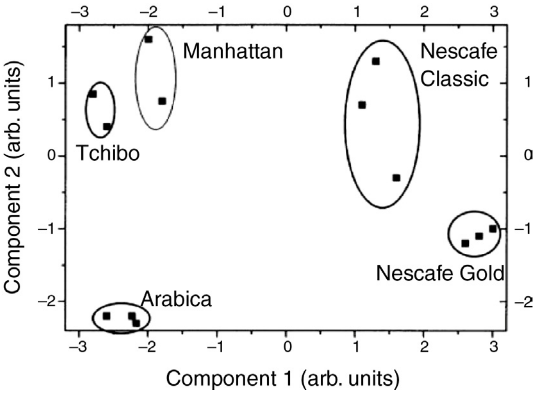

**FIGURE 17.2** PCA plot of different branches of natural and instant (Nescafe) coffees analyzed by an e-tongue.

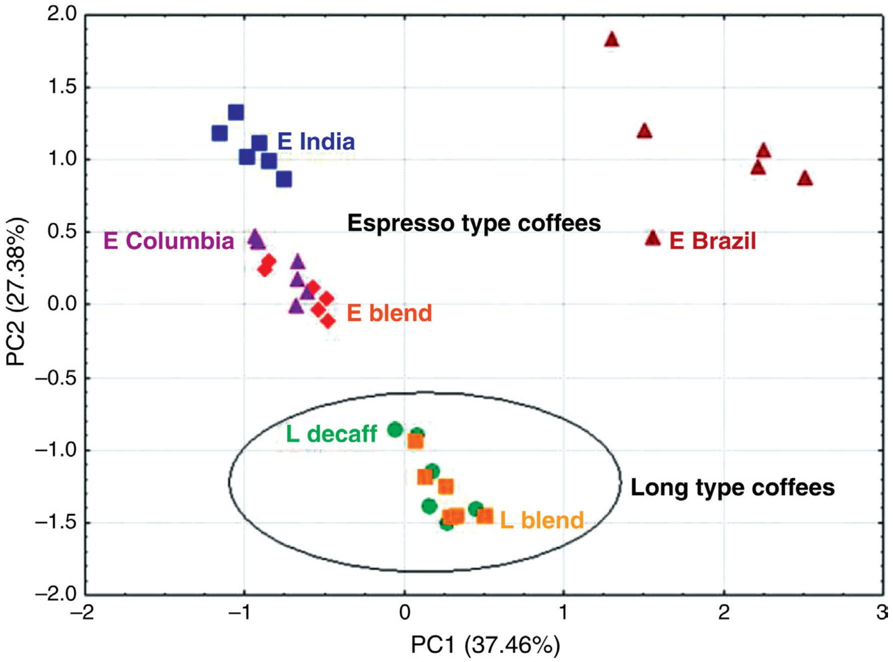

**FIGURE 17.3** PCA plot for the data of espresso and long type coffee samples analyzed by an e-tongue (PC2–PC1).

seven modified ISFET (ion sensitive–field effect transistor) potentiometric transducers with different organic coatings. The coffee samples were originated from Brazil, India, and Colombia (referred to as Columbia in the original paper), besides a blend of these three coffees. Four of the samples were espresso type (coded E Brazil, E India, E Columbia, and E blend), two were of long type (espresso coffee with much more water), and one of which was decaffeinated (coded L blend and L decaff). PCA was used as a statistical tool to reduce the number of variables measured by the seven sensors. Fig. 17.3 shows that the e-tongue was able to distinguish between espresso and long type coffees. It could also separate pure origin from blended coffee. Among the espresso group, E Columbia and E blend samples were found to be similar, consistent with specifications from the manufacturer (Várvölgyi et al., 2015).

### 17.3.2 Detection and Quantification of Specific Substances in Coffee

The evaluation and quantification of specific substances in real samples, including distinct brands of coffee, have been carried out through electrochemical techniques that are simpler, faster, and cheaper. Other characterization methods are chromatography, mass spectrometry, UV–vis and FTIR spectroscopy, and nuclear magnetic resonance (Khoo et al., 2013). Table 17.2 summarizes the literature on the use of electrochemical methods to determine the concentration of caffeine and chlorogenic acid in coffee samples. Results mentioned in Table 17.2 are briefly described in the following paragraphs, where we included figures of merit for the performance of the various sensors, for the sake of comparison.

Pizzariello et al. (1999) developed a potentiometric biosensor based on a glass membrane electrode, which is pH sensitive. For standard samples (not real), the biosensor achieved the following performance: the response was proportional to caffeine concentration between 0 and 4 mg/mL, with a time of analysis between (2 ± 4) min and detection limit of 0.6 mg/L. The potentiometric response of the biosensor was reproducible and up to ten samples could be analyzed per hour. The biosensor performance for caffeine in espresso coffee (real samples) was evaluated comparing its results with those obtained using high-performance liquid chromatography (HPLC). The concentrations of caffeine obtained by both methodologies for decaffeinated, Arabica, and Robusta samples are presented in Table 17.2. Mersal (2012) applied square wave voltammetry to determine caffeine using a pseudo carbon paste microelectrode prepared by mixing graphite powder with paraffin wax. For standard samples, caffeine could be detected in the linear range from $1 \times 10^{-6}$ to $1 \times 10^{-3}$mol/L, with $3.03 \times 10^{-7}$ standard deviation, a correlation coefficient of 0.999, and a detection limit of $3.48 \times 10^{-7}$mol/L. The reproducibility was examined through ten successive measurements in a sample containing $1 \times 10^{-4}$mol/L caffeine, with an observed 0.3% relative standard deviation. The method was applied to determine caffeine concentration in distinct real samples such as tea, coffee, and Coca-Cola.

Square wave voltammetry was used to determine caffeine concentration using a glassy carbon electrode modified with

**TABLE 17.2** Summary of the Literature Devoted to Determine Caffeine and Chlorogenic Acid Concentrations in Coffee Samples Using Distinct Electrochemical Methods

| Coffee Sample | | Concentration | Technique | References |
|---|---|---|---|---|
| Espresso decaffeinated | Caffeine | 0.27 ± 0.04 mg/mL | Potentiometry | Pizzariello et al. (1999) |
| | | 0.35 ± 0.05 mg/mL | HPLC | |
| Espresso Arabica | | 1.00 ± 0.06 mg/mL | Potentiometry | |
| | | 1.41 ± 0.07 mg/mL | HPLC | |
| Espresso robusta | | 2.56 ± 0.12 mg/mL | Potentiometry | |
| | | 2.32 ± 0.15 mg/mL | HPLC | |
| Coffee | | 163 mg/L | Square-wave voltammetry | Mersal (2012) |
| Instant coffee of Nescafe Sunrise | | 96.44 ± 0.03 mg/gm | Square-wave voltammetry | Gupta et al. (2013) |
| Soluble coffee | | 82 mg/L | Differential pulse voltammetry | Khoo et al. (2013) |
| Sachet of Nescafé | | 229.5 μM | Differential pulse voltammetry (bare GCE) | Carolina Torres et al. (2014) |
| | | 220.3 μM | Differential pulse voltammetry (Nafion/GCE) | |
| | | 233.2 μM | Labeled | |
| Instant coffee | | 64.1±2.5 mg/L | Differential pulse adsorptive stripping voltammetry | Tyszczuk-Rotko and Beczkowska (2015) |
| | | 65.5 ± 1.9 mg/L | Spectrophotometry | |
| Coffee | | 25.50 ± 1.82 mg/g | Square-wave adsorptive stripping voltammetry | Yardım et al. (2013) |
| Vacuum–packed, roasted, and ground coffee (strong A) | Chlorogenic acid | 446.7 ± 0.1 mg/L | Square-wave voltammetry | de Carvalho et al. (2008) |
| | | 444.8 ± 0.1 mg/L | Capillary electrophoresis method | |
| Vacuum–packed, roasted, and ground coffee (strong B) | | 544.3 ± 0.1 mg/L | Square-wave voltammetry | |
| | | 545.0 ± 0.1 mg/L | Capillary electrophoresis method | |
| Vacuum-packed, roasted, and ground coffee (traditional C) | | 552.8 ± 0.1 mg/L | Square-wave voltammetry | |
| | | 522.7 ± 0.1 mg/L | Capillary electrophoresis method | |
| Vacuum–packed, roasted, and ground coffee (traditional D) | | 755.0 ± 0.1 mg/L | Square-wave voltammetry | |
| | | 746.5 ± 0.1 mg/L | Capillary electrophoresis method | |
| Coffee | | Add: 3.63 μg/mL<br>Found: 3.7 ± 0.1 μg/mL | Differential pulse voltammetry (standard addition method) | Santos et al. (2011) |
| | | Add: 9.08 μg/mL<br>Found: 8.9 ± 0.1 μg/mL | | |
| | | Add: 10.89 μg/mL<br>Found: 10.5 ± 0.2 μg/mL | | |

multiwall carbon nanotubes (Gupta et al., 2013). For standard samples, the sensor was able to determine caffeine in a wide concentration range, from 10 to 500 μmol/L, reaching a detection limit of $3.52 \times 10^{-3}$mol/L, limit of quantification of $11.73 \times 10^{-3}$ μmol/L, and sensitivity of 48.54 μA $(\mu\text{mol/L})^{-1}$. The method was then applied to determine caffeine concentration in real samples such as tea leaves (Mohani tea leaves), coffee (instant coffee of Nescafe Sunrise), cold drink (Mountain Dew), pharmaceutical preparations, and urine samples.

The concentration of caffeine in soluble coffee, teas, and energy drinks could be determined without any sample pretreatment with differential pulse voltammetry, in which the modified electrodes were made of electrochemically reduced graphene oxide (ERGO; Khoo et al., 2013). The ERGO modified electrode was used in real samples due to its higher sensitivity to the oxidation of caffeine in standard samples, compared to electrodes prepared with graphite oxide (GPO) or graphene oxide (GO). The concentration range at which the sensors could be used to determine caffeine in standard samples was between 50 and 300 μmol/L. In a similar work based on differential pulse voltammetry, Carolina Torres et al. (2014) detected caffeine using a glassy carbon electrode (GCE) with the surface modified with poly(3,4-ethylenedioxythiophene) (PEDOT), Nafion, or multiwalled carbon nanotubes. The unmodified GCE presented the best analytical properties. For instance, detection limits were (128 ± 6) and (38.9 ± 3.7) nmol/L, respectively, for GCE modified with Nafion and unmodified electrodes. However, the GCE modified with Nafion was recommended for sensing experiments involving detection of caffeine in real samples due to its ability to minimize ascorbate interference. In this case, the effect of interfering compounds, such as ascorbic acid, glucose, sucrose, and fructose, usually found in samples containing caffeine, was investigated by both bare GCE and GCE modified with Nafion. These two electrodes were then applied to determine caffeine concentration in three pharmaceutical preparations (tablets of Ilvico, Gurosan, and Dolviran) and three types of beverages (sachet of Nescafé, Coca-Cola, and Redbull). The results for coffee are given in Table 17.2, which also contain the labeled values for the commercial products.

The differential pulse adsorptive stripping voltammetry is another technique exploited to determine caffeine concentrations, where the working electrode consists of a glassy carbon electrode modified with a lead film recovered by a Nafion layer (Tyszczuk-Rotko and Bęczkowska, 2015). For standard samples, the calibration graphs were linear from $5 \times 10^{-8}$ to $5 \times 10^{-6}$mol/L (peak 1 = 0.86 V) and from $5 \times 10^{-7}$ to $1 \times 10^{-5}$mol/L (peak 2 = 1.40 V). The correlation coefficients ($R^2$) were 0.9997 (peak 1 = 0.86 V) and 0.9999 (peak 2 = 1.40 V), and the detection limits were $1.7 \times 10^{-8}$mol/L (peak 1 = 0.86 V) and $2.2 \times 10^{-7}$mol/L (peak 2 = 1.40 V). The electrode-to-electrode reproducibility was verified for three modified electrodes prepared independently and a relative standard deviation of 4.2% was obtained for measurements performed for a $1 \times 10^{-6}$mol/L caffeine solution. The method was then applied to determine caffeine concentration in 10 commercially available caffeine beverages. The results for coffee could be compared with data obtained using UV–vis absorption spectroscopy.

As for determination of chlorogenic acid in coffee samples, we were able to find two papers dedicated specifically for this task. de Carvalho et al. (2008) used square wave voltammetry with a modified carbon paste electrode, obtained by mixing a tetranuclear copper(II) complex to graphite powder and then mineral oil. The tetranuclear copper(II) can mimic the active site of catechol oxidase complex, thus leading to a biomimetic sensor. For standard samples, the latter presented a linear regime in the concentration range from $5.0 \times 10^{-6}$ to $1.45 \times 10^{-4}$mol/L ($r = 0.9985$), with a detection limit of $8.0 \times 10^{-7}$mol/L. The biomimetic sensor was very stable (250 days; 640 determinations) and reproducible with relative standard deviation of 10.0%. For real samples, four kinds of coffee (vacuum-packed roasted and ground coffee) obtained from local supermarkets were analyzed in triplicate. The results from the biomimetic sensor, illustrated in Table 17.2, were compared to those obtained with the capillary electrophoresis method.

In the other paper, differential pulse voltammetry was used with a molecularly imprinted sensor (Santos et al., 2011). The sensor was assembled by depositing an imprinted siloxane film, prepared by sol–gel process, onto an Au bare electrode surface. For standard samples, the sensor presented selectivity toward chlorogenic acid, a linear response (peak current) from $5.0 \times 10^{-7}$mol/L to $1.4 \times 10^{-5}$mol/L, and a detection limit of $1.48 \times 10^{-7}$mol/L. Four real samples, including coffee, black tea, green tea, and mate tea, were analyzed. The results for coffee are given in Table 17.2.

Caffeine and chlorogenic acid were determined simultaneously using cyclic and adsorptive stripping voltammetry, with a boron-doped diamond electrode (Yardım et al., 2013). The limits of detection for standard samples were 0.107 mg/mL ($5.51 \times 10^{-7}$mol/L) for caffeine and 0.448 mg/mL ($1.26 \times 10^{-6}$mol/L) for chlorogenic acid. The practical application of this method was tested in commercially available beverage samples such as coffee and energy drinks using the spike/recovery method. The concentrations found for caffeine and polyphenol content in coffee, energy drinks, and cola were also determined. According to Yardım et al., the results are within the legal limits established by the Turkish Food Codex and agree with the amount displayed on the label by the manufacturer (coffee: 25–54 mg/g; beverages: ≤ 150 mg/L).

## 17.4 ELECTRONIC TONGUES USING ELECTRICAL IMPEDANCE

Impedance spectroscopy has been an important tool to investigate mass transport, bulk polymers, biological and interfacial effects, and surface corrosion (Barsoukov and Macdonald, 2005). It has also been used in e-tongues (Riul et al., 2003; Smyth and Cozzoino, 2013), some of which were applied in coffee analysis (Riul et al., 2003; Ferreira et al., 2007). These e-tongues had sensing units made with ultrathin films of distinct materials deposited onto gold interdigitated electrodes (IDEs). Some advantages of e-tongues based on impedance spectroscopy are that they have no need of a reference electrode and elevated sensitivity to electrolytes and nonelectrolytes due to the ultra-thin nature of the transducers. The drawbacks are mainly associated with the need of computational methods to readjust the setup when a sensing unit has to be replaced.

An e-tongue based on impedance spectroscopy was able to distinguish 36 coffee samples having distinct global quality (GQ) scores assigned by trained coffee tasters. With regression models proposed in the machine learning area, it was possible to predict GQ within ± 0.3 when compared with expert human tasters, quite promising for coffee analysis (Ferreira et al., 2007).

Microfluidic devices began to appear recently (Jacesko et al., 2005; Daikuzono et al., 2015), paving the way for integrations in clinical, environmental, foodstuff, and pharmaceutical applications. An impedance-based microfluidic e-tongue containing an array of sensing units was especially designed to receive layer-by-layer (LbL) films inside the microchannels, which are made with polydimethylsiloxane (PDMS) sealed onto gold IDEs (Daikuzono et al., 2015). The films were adsorbed by sequentially passing polyelectrolytes into the microchannels. After deposition of the multilayers, the individual sensing units could be easily integrated, as illustrated in Fig. 17.4. This offers a series of advantages, including the use of only microliters for sampling and discharge and a large decrease in the time of analysis. Most importantly, it is possible to exploit such easy integration for producing sensor arrays with tailored multiple functionalities.

Further experimental details of the impedance-based microfluidic e-tongue are as follows. The PDMS microchannels were 490 μm wide, 50 μm high, and 12.5 mm long, being sealed onto gold IDEs (30 pairs of fingers, 40 μm wide, 3 mm long, and 40 μm apart from each other) using plasma oxygen. The microchannels and IDEs were fabricated at the Brazilian Nanotechnology National Laboratory (LNNano). The LbL films were obtained from polyallylamine chloride (PAH) in the cationic layers, while the anionic layers consisted of poly(3,4-ethyenedi oxythiophene):poly(styrenesulfonate) (PEDOT:PSS), or polypyrrole (PPy) or nickel phthalocyanine (NiTsPc). In each microchannel a 5-bilayer LbL film was deposited, thus forming the e-tongue setup. As a proof-of-principle, the microfluidic e-tongue was used to distinguish electrolyte and nonelectrolyte substances, corresponding to the basic tastes (Daikuzono et al., 2015).

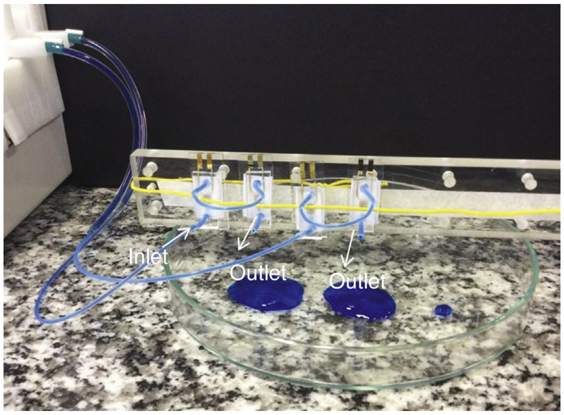

FIGURE 17.4 Experimental setup integrating individual sensing units of a microfluidic impedance based e-tongue.

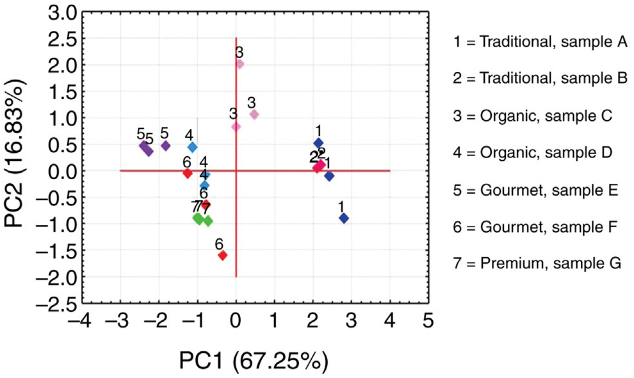

FIGURE 17.5 PCA plot for the data obtained with Brazilian coffees of distinct global quality features analyzed by a microfluidic e-tongue, with measurements taken at 5 kHz.

In addition, this microfluidic e-tongue was employed to analyze Brazilian coffees obtained at local supermarkets. The samples were chosen due to their distinct characteristics of quality and production. Fig. 17.5 shows the PCA plot for the data taken in triplicate at 5 kHz for seven distinct coffees, using ~200 μL for each sample. The frequency was chosen in order to avoid interfacial effects that occur at lower frequencies and the resonance in the circuit due to the geometry of the IDEs used. Coffees with similar quality features were grouped closed to each other and similar samples displayed score values with the same sign indicating that most of the variances observed are attributed to physical characteristics moving the data in the same way throughout PC1.

Considering the simplicity involved in such analysis, namely the use of only four sensing units and avoiding prohibitive costs of a trained panel of human experts, the system brings all benefits from microfluidics. The latter include low cost, small amounts of samples, and the possibility to fabricate lab-on-a-chip devices. One can now envisage running complex analytical systems with high efficiency, paving the way for future developments and applications.

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## Chapter 18

# Gliadins in Foods and the Electronic Tongue

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## 18.1 INTRODUCTION

Food allergy is an adverse, abnormal immune-mediated reaction to a certain food or food ingredient that appears in susceptible individuals, often requiring a strict avoidance of their ingestion (Amaya-González et al., 2013). Sometimes, people exhibit food sensitivity, including intolerance that is a nonimmune-mediated reaction. The incidence of these disorders is difficult to assess and the percentage of people self-perceived as food intolerant (up to 25%) is very different from that of confirmed cases (less than 3%). Most allergens are proteins that must be detected along the food chain, posing a real challenge for the development of analytical methods. Gliadin is a heat-stable allergen, known as the alcohol-soluble fraction of gluten, being the antigenic protein of wheat responsible for celiac disease. The ratio of gliadin to total gluten varies with the food matrix. The gliadin content usually corresponds to half of the gluten content (Peres et al., 2011), although this value is not consensual (EFSA, 2004; Tranquet et al., 2012). A daily intake of 100 mg of gliadin can induce clinical symptoms in celiac patients, being the prevalence of celiac disease (classical, oligosymptomatic, and silent forms) in children and adults around 1:200 in Europe (EFSA, 2004). Therefore, a threefold definition of gluten-free foods was proposed (EFSA, 2004): (1) foods in which ingredients do not contain any prolamin from wheat or *Triticum* species with a gluten level not exceeding 20 mg/kg (or ppm); (2) those consisting of ingredients which have been rendered "gluten-free" with a gluten level not exceeding 200 mg/kg; and (3) those resulting from a mixture of ingredients with a gluten level not exceeding 200 mg/kg. It should be noticed that the values previously mentioned are only indicative since there is not enough information to make a final decision on them. More recently, foods labeled as "very low gluten" or "gluten-free" must have gluten content lower than 100 and 20 mg/kg, respectively (Nassef et al., 2008; OJEU, 2009; Zeltner et al., 2009). However, commercial foods labeled as gluten-free may be contaminated by gluten in the range of 20–200 ppm (Collin et al., 2004; Scognamiglio et al., 2014).

Several commercial analytical tools have been developed, namely to detect gliadin/gluten, most of them relying in immunoassays, both competitive for hydrolyzed food and sandwich formats for complete proteins (Amaya-González et al., 2013). Moreover, emerging electrochemical techniques such as aptasensors and electronic tongues (e-tongues) have also been reported (Amaya-González et al., 2014; Meirinho et al., 2015; Peres et al., 2011).

## 18.2 GLIADINS AND THE CELIAC DISEASE

The celiac disease is classified as an autoimmune disease of the small intestine induced in genetically susceptible individuals that is caused by the ingestion of gluten proteins, which are important components of commonly used food sources like wheat, rye, and barley (Bai et al., 2013; Shan et al., 2002). The introduction of gluten-rich foods in the human diet led to the development of disease related to gluten exposure (Sapone et al., 2012; Troncone and Jabri, 2011). These reactions are not restricted to celiac disease, but also include nonceliac gluten sensitivity and wheat allergy, which combined affect about 10% of the general population (Battais et al., 2003, 2005; Lammers et al., 2014; Williams et al., 2010; Woodward, 2010). Moreover, they represent distinct pathophysiological reactions to gluten ingestion, with differing clinical presentations, serological markers, and long-term treatments (Bai et al., 2013; Briani et al., 2008; Ciclitira et al., 2005b). Although current

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research attempts to elucidate the frontiers between these reactions, their differences can be difficult to discriminate.

In the case of celiac patients, the exposure to gluten induces an inflammatory response that ultimately will lead to the destruction of the villous structure of the intestine (Shan et al., 2002; Williams et al., 2010; Woodward, 2010). It usually appears in early childhood with pronounced symptoms such as chronic diarrhea, abdominal distension, and failure to thrive. In some patients, symptoms are only revealed later in life and these may include fatigue, diarrhea, and weight loss due to malabsorption, anemia, and neurological symptoms (Ciclitira and Moodie, 2003). Celiac disease is a life-long disease and if untreated, it is associated with increased morbidity and mortality. Despite its high prevalence and severe symptoms, the only effective therapy is a strict dietary abstinence from the previously mentioned food grains (Briani et al., 2008; Fric et al., 2011; Tye-Din et al., 2010; Sapone et al., 2012).

Gluten is the main structural protein complex present in wheat with equivalent toxic proteins found in rye and barley (Sapone et al., 2012). The amino acid composition of gluten peptides with a high percentage of glutamine (up to 35%) and proline (15–20%) is unique (Fric et al., 2011). Immune-reactive protein fractions of gluten comprise gliadins and glutenins, with gliadins containing monomeric proteins and glutenins containing aggregated proteins (Bittner et al., 2008). Gliadins are complex glycoproteins rich in proline and glutamine (Lammers et al., 2014). Due to their structure, the intestinal enzymes cannot completely degrade the proteins. Actually, it is well known that undigested or partly digested gliadins can affect a broad range of human cells (eg, inhibit cell growth, induce apoptosis, and alter redox equilibrium).

The celiac disease is a model autoimmune disease, in which, contrarily to many other autoimmune diseases, the trigger (gluten), the tight genetic junction (HLA antigens: DQ2 and DQ8), as well as the primary autoimmune reaction [autoantibodies to tissue transglutaminase (tTG)] are known (Fric et al., 2011; Sollid et al., 2012; Tye-Din et al., 2010). This knowledge represents an advantage in the development of new diagnosis and treatment methods, as well as for the development of food analytical techniques that can easily and accurately detect the presence of gluten-related toxic protein fractions, such as gliadins. Indeed, the main clinical issues in the management of celiac disease are that the diagnostics are suboptimal and invasive, and that patients must rely on a complex, costly, and life-long therapy (Tye-Din et al., 2010). While intestinal biopsy is still considered the gold standard for diagnosing celiac disease, the presence of highly specific autoantibodies in patient serum has been clinically used as a marker for screening candidates for duodenal biopsy (Bizzaro et al., 2012; Ciclitira and Moodie, 2003; Williams et al., 2010; Woodward, 2010). Additionally, the relevance of antibody assessment in predicting celiac disease has increased along with the number of patients with minor or atypical symptoms.

As previously mentioned, from the human diseases related to gluten exposure, the best known are mediated by the adaptive immune system and include celiac disease and wheat allergy (Battais et al., 2003). In both conditions, the reaction to gluten is mediated by T-cell activation in the gastrointestinal mucosa (Han et al., 2013; Sapone et al., 2012). However, in wheat allergy, it is the cross-linking of immunoglobulin IgE by repeat sequences in gluten peptides that triggers the release of chemical mediators. Contrarily, the celiac disease is an autoimmune disorder as demonstrated by specific serologic autoantibodies [tTG and antiendomysium antibodies (EMA)] (Han et al., 2013). Besides these two conditions, there are cases of gluten reactions in which neither allergic nor autoimmune mechanisms are involved. These are generally defined as gluten sensitivity. Individuals exhibiting gluten sensitivity are unable to tolerate gluten and develop an adverse reaction different from the one observed in patients with celiac disease, that is, without damage in the small intestine (Bai et al., 2013; Troncone and Jabri, 2011). Although the symptoms may be similar to those associated with celiac disease, no tTG autoantibodies or other specific celiac-related antibodies are found.

Although a gluten-free diet is prescribed to patients suffering from diseases related to gluten exposure, this does not mean that they cannot tolerate gluten at all, as their clinical sensitivity varies significantly (Ciclitira et al., 2005a; Hischenhuber et al., 2005). Some individuals cannot tolerate trace amounts of gluten, whereas others appear to tolerate large amounts. In the standard Western European gluten-free diet, some gluten is accepted as a contaminant in wheat starch (Kupper, 2005). This starch improves the baking quality and palatability of the gluten-free diet and it is tolerated by most celiac patients (Collin et al., 2004; Fido et al., 1997; Goesaert et al., 2005; Peraaho et al., 2003). In other countries, such as United States of America, for example, wheat starch is not recommended. The US National Food Authority has decided that the label "gluten-free" can only be used for foods that contain no gluten at all (Kupper, 2005). Therefore, foods that contain wheat starch should be labeled as "low-gluten" (Fasano and Catassi, 2001). The proposed standard as formulated by the WHO/FAO organization *Codex Alimentarius*, has one limit at 0.02% for "rendered gluten-free" food, and another at 0.002% for "naturally gluten-free food" (Bai et al., 2013; Hischenhuber et al., 2005; Niewinski, 2008). These different practices reflect the fact that we do not know the exact limit of gluten intake that is tolerated long term without harmful effects by patients with celiac disease as a group (Ciclitira et al., 2005a). The acceptable gluten dose per day is still under debate, although the scientific and medical communities have suggested that around 50 mg/day is safe (Hischenhuber et al., 2005). As a reference, the normal gluten intake by healthy individuals is about 13 g/day (Van Overbeek et al., 1997). Research indicates no significant differences in susceptible individuals undergoing a strict wheat starch-containing, gluten-free diet versus a naturally gluten-free diet (Kupper, 2005).

In summary, the awareness of the gluten-related diseases, its dietary restrictions, and the impact of adhering to a gluten-free diet warrant further research. Also, reliable detection and quantification methods for food allergens, such as gluten proteins, are required to ensure compliance with food labeling and to improve consumer protection.

## 18.3 SENSOR DEVICES FOR GLIADIN AND/OR GLUTEN DETECTION IN FOODS

The availability of fast, sensitive, and reliable analytical methods to detect specific food risks, ensuring food safety for people susceptible or intolerant to some food substances that may be allergens like gliadins is of huge importance and a real need. Indeed, in a recently market survey carried out in the United States (Sharma et al., 2015), it was reported that 3.6% of the gluten-free labeled foods evaluated contained 5.8–554 ppm of gluten, and 1.1% of those foods had gluten contents greater than the regulatory threshold (20 ppm). The limitations (eg, nonportability; strict operating conditions; and required highly qualified trained technicians) associated to the high-cost and time-consuming traditional methods (eg, gel or capillary electrophoresis, high-performance liquid chromatography, polymerase chain reaction) have encouraged the development of emerging sensor-based technologies. Nevertheless, it should be stated that those traditional techniques are complementary and sensitive tools that are commonly used to confirm the results of the immunological officially accepted methods (Rosell et al., 2014).

### 18.3.1 Factors Affecting Gliadins/Gluten Analysis

There are two issues that can make gluten analysis difficult, namely, the extraction yield of gliadin from the food samples and the use of a correct gliadin standard. These two factors may limit the development and/or implementation of novel analytical approaches for gluten-free food analysis (Rosell et al., 2014). Gluten extraction from processed foods is not an easy task, since, in some cases, during the food processing, high temperatures are used that contribute to the formation of isopeptide bonds between amino and carboxamide groups of the protein residues or to the formation of protein aggregates making gluten analysis quite difficult. Therefore, to ensure a complete extraction of both prolamins and glutenins, several cocktail recovery solutions have been proposed (Garcia et al., 2005; Mena et al., 2012), although some of them, namely those using β-mercaptoethanol, may be incompatible with some immunological-based techniques. On the other hand, in gluten analysis, the use of the most adequate standard plays an important role. The standard should be as representative as possible of the gluten proteins to be analyzed. Some standards are available, such as *The Working Group on Prolamin Analysis and Toxicity* (PWG) gliadin standard (Van Eckert et al., 2006). Nevertheless, its use is not consensual. Some authors have suggested that it would be more correct to use a hydrolyzed standard combined with a competitive assay to quantify peptides of partially hydrolyzed gluten in fermented wheat, rye, and barley products (Comino et al., 2012, 2013; Gessendorfer et al., 2009; Mena et al., 2012; Rosell et al., 2014).

Regardless of these pertinent questions, several works have reported the development of sensor-based analytical methodologies toward the detection of gliadins in foodstuffs using commercial gliadin or gluten standards and extraction approaches based on the use of aqueous–ethanolic solutions.

### 18.3.2 Immuno- and Aptasensors for Gliadin/Gluten Detection in Foods

In recent years, several optical and electrochemical biosensors, including immunosensors and aptasensors, have been developed to detect gliadin in food matrices, namely to evaluate gluten-free foods, since the amount of gluten must be lower than 20 mg/kg (or ppm), according to the legal requirements (Nassef et al., 2008; OJEU, 2009; Zeltner et al., 2009). De Stefano et al. (2006) used an optical sensor with a recombinant glutamine-binding protein to detect traces of gluten in food. Nassef et al. (2008) proposed an electrochemical immunosensor, based on the use of an antibody raised against the putative immunodominant celiac disease epitope, to measure the gliadin content in foods. Detection limits between 5.5 and 11.6 ng/mL (or ppb) could be achieved. Labelless impedimetric and antigliadin Fab-based amperometric immunosensors were also developed by Nassef et al. (2009), showing gliadin detection limits of 3.23 ng/mL. Mairal et al. (2009) developed a microfluorimeter with a disposable polymer chip with a gliadin detection limit of 4.1 ng/mL, by detecting the emission of a fluorophore-labeled monoclonal antigliadin antibody upon excitation with light. Laube et al. (2011) developed an electrochemical magneto immunosensor, coupled or not to ELISA, allowing the quantification of gliadin or small gliadin fragments in natural or pretreated food samples with detection limits ranging from 1.2 to 24.2 ng/mL (depending on the food matrix). Chu and Wen (2013) developed a sensitive liposomal fluorescence immunoassay with immunomagnetic beads for the detection and quantification of gliadin in gluten-free foods with a detection limit of 0.6 µg/mL (or ppm), although slight cross-reactions with barley and rye were found. Although the immunosensor technology seems promising, limitations like long-term stability, surface effects, and interferences resulting from complex sample matrices are major concerns (Neves et al., 2010). Also, finding a single antibody able to react with different gliadin and glutenin subunits with similar affinity, as well as with prolamins from different cereals and from modified gluten is very challenging (Tranquet et al., 2012). Thus, due to the complexity of gluten proteins, the quantification of the total gluten content in foods is extrapolated from the gliadin concentration, assuming a constant gliadin–glutenin ratio equal to 1 within all samples (Tranquet et al., 2012). Gluten composition depends on multiple parameters such as the species, cultivars, agronomical conditions, as well as on the products processing (Wieser and Koehler, 2009). Therefore, some authors (Van Eckert et al., 2010; Wieser and Koehler, 2009) have suggested that the next step could be the use of a mixture of antibodies that could recognize gliadin and glutenin subunits at similar degrees. Still, the development of such assays, with two or more antibodies, is complex and may be expensive (Tranquet et al., 2012).

Hence, recently aptamers against hydrophobic immunotoxic peptides from gliadin from wheat that also recognize celiac disease related proteins from barley, rye, and oat have been investigated (Amaya-González et al., 2013; Pinto et al., 2014). Fernández et al. (2012) developed an electrochemical genosensor for the detection of a specific DNA sequence that encodes an immunogenic fragment of gliadin, being achieved a detection limit of 0.001 μM. More recently, Amaya-González et al. (2014) reported a competitive electrochemical magneto-assay without cross-reactivity with nontriggering celiac disease proteins from soya, rice, or maize. This device enabled the detection of 0.5 ppb in diluted gliadin standard solutions, which corresponds to a detection limit of 0.5 ppm of gluten, considering the dilution factor and assuming that gliadin constitutes 50% of gluten.

### 18.3.3 Electronic Tongue

Although optical and electrochemical immuno- and aptasensors proved to be a potentially fast and practical tool to accurately detect possible gliadin/gluten contamination of gluten-free labeled foods, their development has been very demanding, requiring a considerable amount of consumables, equipment, and skilled technicians. A possible and simpler alternative has been proposed by Peres et al. (2011) and is based on the use of an all solid-state potentiometric e-tongue with 36 polymeric membranes, not coupled to any antibody against gliadin, or aptamer against any immunotoxic peptides from gliadin. The device comprised two-sensor arrays, being the membranes prepared with organic compounds containing long carbon chain with different functional groups (lipid additive compounds). Each membrane contained polyvinyl chloride (PVC) as polymeric matrix, a plasticizer, and a sensor additive. The multisensor device enabled the semiquantitative discrimination of aqueous–ethanolic (30:70, v/v) mixtures, containing pre-established levels of dissolved gliadin standard, chosen in order to mimic food aqueous–ethanolic extracts of gluten-free, low-gluten content, or gluten-containing foodstuffs (<20; 20–200; and >200 mg/kg of gluten equivalent, assuming a gliadin/gluten ratio equal to 0.5) with a sensitivity of around 80%, corresponding to a gliadin detection limit around 1–2 mg/kg. Also, e-tongue was successfully applied to real samples, being able to correctly classify more than 80% of the gluten-free or gluten-containing foodstuffs evaluated.

The successful performance reported by the research team (Peres et al., 2011) may be tentatively explained based on the chemical composition of the polymeric membranes applied on the e-tongue. Indeed, the lipid polymeric membranes used contain hydrophobic and hydrophilic groups allowing the interaction with several chemical compounds (electrolytes and nonelectrolytes) via electrostatic or hydrophobic interactions (Kobayashi et al., 2010; Toyota et al., 2011a,b; Yasuura et al., 2014a,b). Hydrogen bonds or electrostatic interactions may also arise in the presence of mediating electrolyte substances, between carboxyl or phosphate groups in the lipid/polymer membrane and vicinal hydroxyl groups of the target molecules (Toyota et al., 2011b). Furthermore, it is accepted that lipids interact with proteins during gluten formation; thus, lipids could enhance the formation of large complex aggregates involving both gliadin and glutenin proteins (Carcea and Schofield, 1996). Besides, it is known that nonpolar lipids can be associated with glutenins through either hydrophobic interactions or hydrogen bonds, whereas polar lipids containing phosphate groups preferentially interact with gliadin (McCann et al., 2009). Moreover, when lipid/polymer membranes are applied for protein detection, namely gliadin, that does not bind directly, it is expected that their behavior could mimic that of protein–lipid interactions occurring in biological membranes, where unspecific hydrophobic association or electrostatic interactions between protein and lipid head groups occur (Thomas and Glomset, 1999; Zhao and Lappalainen, 2012).

To further evaluate and verify the possible interaction of the lipidic membranes toward gliadin or gluten from wheat, a new e-tongue was built. The electrochemical device consisted of a print-screen potentiometric array (Fig. 18.1), with 20 chemical sensors, with cross-sensitivity lipidic membranes and relative plasticizer-additive compositions (Table 18.1) identical to those previously used by the research team for gliadin qualitative and semiquantitative detection (Peres et al., 2011). Plasticizer bis(2-ethylhexyl) phthalate was replaced by dioctyl phenylphosphonate and the additives bis(2-ethylhexyl)phosphate and tridodecylmethylammonium chloride were not included in the new

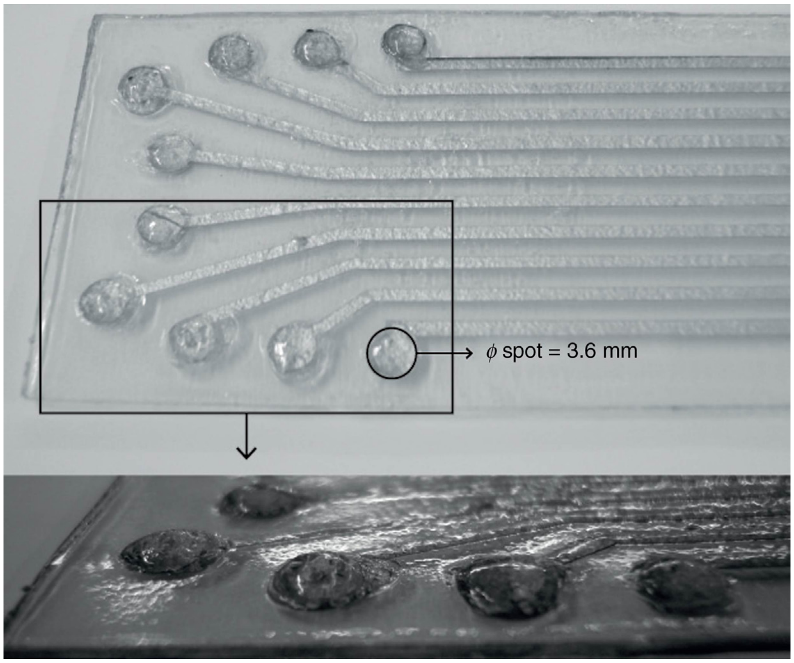

**FIGURE 18.1** **Screen-printed scheme with conductive resin silver of the e-tongue multisensor device, containing 20 lipid/polymeric membranes, used for potentiometric analysis of aqueous–ethanolic gliadin or gluten standard solutions (surface isolated with acrylic resin).**

electrochemical device tested, since in a preliminary study sensors containing those compounds showed low response toward gliadin and other proteins (data not shown). Finally, each lipidic membrane contained PVC (≈32%), as the polymeric matrix, and a combination of each of the five plasticizers used (≈65%) and the four additive compounds (≈3%), as shown in Table 18.1. An identification code was used for each lipidic membrane, containing the letter S as the sensor followed by two numbers (the first identifying the plasticizer, from 1 to 5, and the latter the additive, from 1 to 4) separated by a punctuation mark (comma).

The e-tongue signal profiles were recorded in alkaline aqueous–ethanolic solutions (pH≈12; 30:70 v/v) containing standard gliadin (from Sigma-Aldrich) or gluten (from Sigma-Aldrich, protein content >80%), varying from +86.1 to +151.0 mV and +82.7 to +142.4 mV, respectively. In general, for all sensors, the corrected signal potential $[\Delta E(\text{mV}) = E^0_{\text{dissolved protein}} - E^0_{\text{solvent}}]$ increased with the

**TABLE 18.1** Sensors Used in the E-Tongue: Identification of the Plasticizer and Additive Compounds Used in Each Lipidic-Polymeric Membrane

| ID No.[a] | Name | Chemical Formula |
|---|---|---|
| **Plasticizer compound**[b] | | |
| 1 | Bis(1-butylpentyl) adipate | $[\text{-}(CH_2)_2COOCH[(CH_2)_3CH_3]_2]_2$ |
| 2 | Dibutyl sebacate | $[\text{-}(CH_2)_4CO_2(CH_2)_3CH_3]_2$ |
| 3 | 2-Nitrophenyl-octyl ether | $O_2NC_6H_4O(CH_2)_7CH_3$ |
| 4 | Tris(2-ethylhexyl) phosphate | $[CH_3(CH_2)_3CH(C_2H_5)CH_2O]_3P(O)$ |
| 5 | Dioctyl phenylphosphonate | $C_6H_5P(O)[O(CH_2)_7CH_3]_2$ |
| **Additive compound**[c] | | |
| 1 | Octadecylamine | $CH_3(CH_2)_{17}NH_2$ |
| 2 | Oleyl alcohol | $CH_3(CH_2)_7CH{=}CH(CH_2)_7CH_2OH$ |
| 3 | Methyltrioctylammonium chloride | $[CH_3(CH_2)_6CH_2]_3N(Cl)CH_3$ |
| 4 | Oleic acid | $CH_3(CH_2)_7CH{=}CH(CH_2)_7COOH$ |

[a] *Sensor identification number.*
[b] *All plasticizers were Selectophore*$^{TM}$ *grade from Fluka, with purity ≥97%.*
[c] *All additives were from Fluka, with purity ≥97%.*

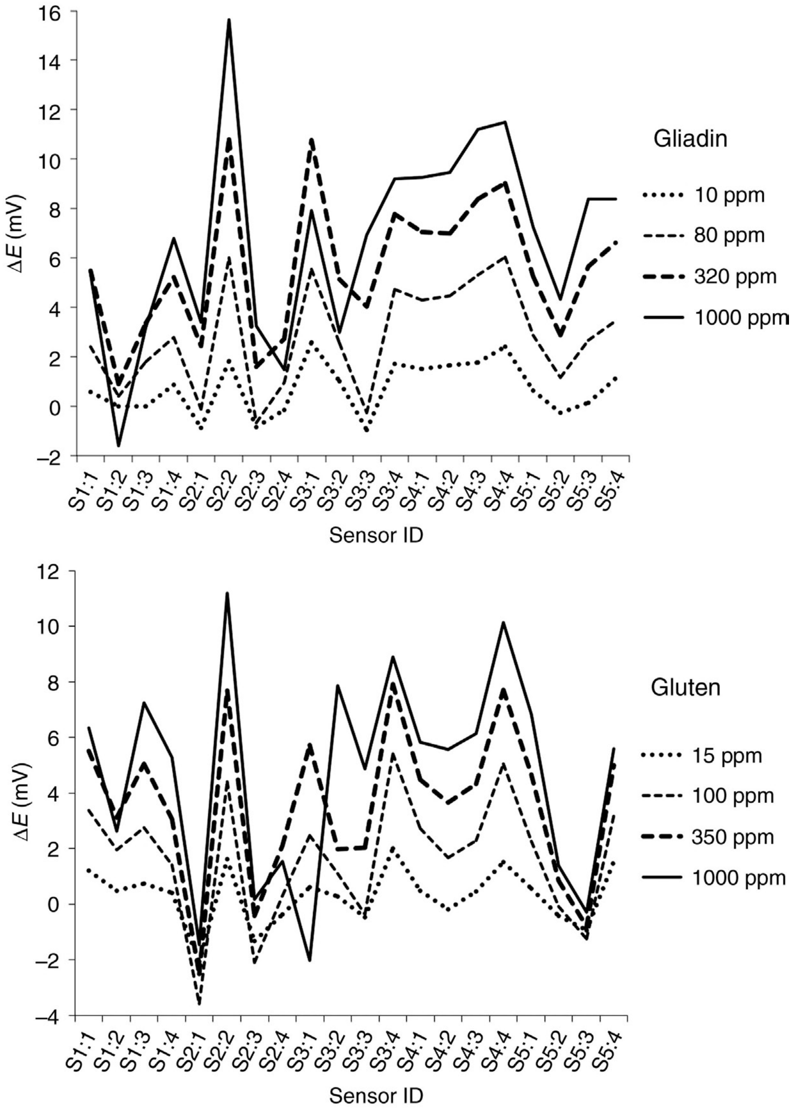

**FIGURE 18.2 The e-tongue corrected signal profiles (Δ*E*, mV) variation with increasing concentration levels of gliadin (10–1000 ppm) or gluten (15–1000 ppm) in aqueous–ethanolic standard solutions (pH≈12).**

gliadin or gluten content, because their responses evaluated in dynamic concentration ranges as can be seen in Fig. 18.2 (gliadin: 10–1000 ppm; gluten: 15–1000 ppm).

Linear correlations were obtained by plotting the sensors' signals against decimal logarithm of the concentrations [$\Delta E(\text{mV}) = a + b \times \log_{10}(C, \text{ppm})$], although for different concentration intervals (Table 18.2 and Fig. 18.3). All sensors showed a quantitative response toward gliadin concentration ($0.967 \leq R \leq 0.997$) enabling the quantification of gliadin in standard solutions. For more complex matrices, such as foodstuffs, the possible quantification of gliadin content must be experimentally evaluated, but it is expected that the use of multivariate regression models (linear or nonlinear) based on a subset of the most informative sensors (chosen using a heuristic or a metaheuristic variable selection algorithm) will overcome possible modeling difficulties, namely due to signal interferences (Dias et al., 2014).

The results clearly show the capability of the e-tongue to quantify gliadin and, although only standard solutions were analyzed, a potential application to real samples can be foreseen. It should be noticed that, if gluten concentrations were converted into *apparent* gliadin contents (assuming a gliadin–glutenin ratio equal to 1) similar regression equations would be obtained for the dependence of $\Delta E$ with $\log_{10}(C)$, independently if the assays were made with gliadin or gluten solutions, as also exemplified in Fig. 18.3 for two e-tongue sensors (S1:4 and S4:4). This result suggests that, in principle, the lipidic membranes are responding preferentially to gliadin over glutenin proteins. This apparent preference may be tentatively explained taking into account that: (1) glutenin has a greater average molecular weight (70–90 kDa) compared to gliadin (30–50 kDa) (Wieser, 2008), which may favor the gliadin possible adsorption over glutenin and (2) lipidic membranes used preferentially interact with gliadin

**TABLE 18.2** Parameters of the Linear-Logarithm Regressions and Dynamic Concentration Ranges for Each Sensor (S1:1 to S5:4) of the Potentiometric E-Tongue [$\Delta E(\text{mV}) = a + b \times \log_{10}(C, \text{ppm})$]

| | Gliadin | | Gluten | |
|---|---|---|---|---|
| Sensor ID No.[a] | Concentration Range (ppm) | $R$[b] | Concentration Range (ppm) | $R$[b] |
| S1:1 | [10, 320] | 0.967 | [15, 570] | 0.999 |
| S1:2 | [10, 320] | 0.992 | [15, 570] | 0.995 |
| S1:3 | [10, 560] | 0.997 | [15, 1000] | 0.993 |
| S1:4 | [10, 1000] | 0.993 | [15, 1000] | 0.996 |
| S2:1 | [80, 1000] | 0.991 | [100, 820] | 0.989 |
| S2:2 | [36, 1000] | 0.994 | [15, 1000] | 0.996 |
| S2:3 | [80, 1000] | 0.993 | [15, 820] | 0.96 |
| S2:4 | [36, 810] | 0.996 | [15, 820] | 0.997 |
| S3:1 | [3, 810] | 0.982 | [15, 820] | 0.990 |
| S3:2 | [3, 560] | 0.997 | [15, 820] | 0.984 |
| S3:3 | [80, 1000] | 0.992 | [100, 820] | 0.975 |
| S3:4 | [10, 560] | 0.996 | [15, 570] | 0.825 |
| S4:1 | [10, 1000] | 0.997 | [190, 570] | 0.991 |
| S4:2 | [10, 1000] | 0.996 | [190, 570] | 0.974 |
| S4:3 | [3, 1000] | 0.996 | [190, 820] | 0.994 |
| S4:4 | [10, 1000] | 0.999 | [190, 1000] | 0.991 |
| S5:1 | [10, 1000] | 0.995 | [190, 1000] | 0.960 |
| S5:2 | [36, 1000] | 0.995 | [190, 1000] | 0.990 |
| S5:3 | [36, 1000] | 0.994 | [190, 1000] | 0.984 |
| S5:4 | [36, 810] | 0.993 | [190, 1000] | 0.979 |

[a] *Sensor identification code number based on the information given in Table 18.1.*
[b] *Correlation coefficient.*

over glutenin proteins due to their polarity and presence of a phosphate group in some of them (McCann et al., 2009). A more detailed analysis of the results, shown in Table 18.2, indicate that apparently the type of additive has less influence in the potentiometric signal responses than the type of plasticizer. Indeed, two plasticizers [tris(2-ethylhexyl) phosphate and dioctyl phenylphosphonate] gave the best correlations, which was expected due to the presence of the phosphate group that enhances the gliadin–lipid interaction (McCann et al., 2009).

Globally, from the results reported by Peres et al. (2011) and those obtained in this work, both based on the use of potentiometric e-tongues with lipid/polymeric membranes, it can be inferred that this electrochemical approach exhibits a sensitivity of 1–3 ppm (≈2–6 ppm of gluten), which is quite satisfactory since an analytical method with a sensitivity of 10 ppm is suitable for gluten detection (Zeltner et al., 2009). Moreover, the e-tongue fulfills the requirements of gluten-free, low-gluten content, or gluten-containing food label verification, enabling gliadin content quantification in a wide dynamic range, varying from 3 to 1000 ppm. However, this quantitative potential must be further investigated by applying the device to real food samples. Nonetheless, this work together with the previous one (Peres et al., 2011) may be viewed as a proof-of-principle that a potentiometric e-tongue with lipidic membranes may be used as a practical, fast, simple, and sensitive tool toward the detection of gliadin.

## 18.4 CONCLUSIONS AND FINAL REMARKS

Several analytical techniques have been reported for gliadin detection in food samples. Recently, the use of sensors gained an increased attention, namely immunosensors and aptasensors, which exhibit gliadin detection limits (3 ppb–0.6 ppm, depending on the technique) much lower than the regulatory gluten threshold allowed in gluten-free foodstuffs (<10 ppm of equivalent gliadin). Nevertheless, these high-sensitive techniques are usually far beyond the economic and technical possibilities of the majority of the food industries, namely micro- and small familiar enterprises, reducing its routine application. Hence, in recent years the research team has developed electrochemical devices for gliadin detection in food samples. The potentiometric e-tongues developed have exhibited a suitable sensitivity toward gliadin (1–3 ppm) enabling

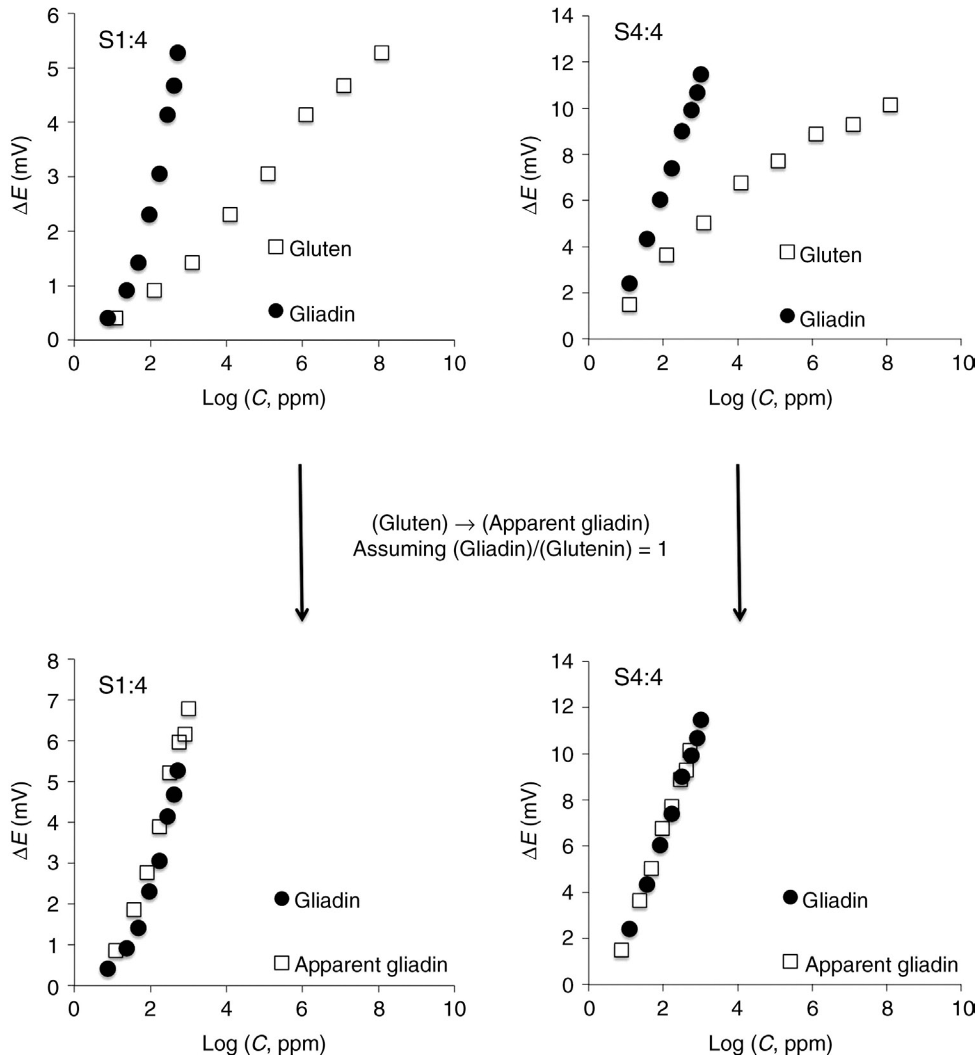

**FIGURE 18.3** The e-tongue calibration: corrected potential signals (Δ*E*, mV) versus decimal logarithm of gliadin or gluten concentration, for two sensors [S1:4 and S4:4, corresponding to a plasticizer/additive combination of bis(1-butylpentyl) adipate/oleic acid and tris(2-ethylhexyl) phosphate/oleic acid, respectively].

its quantification, as well as the qualitative or semiquantitative discrimination of foods based on their gluten content and according to the legal thresholds. The satisfactory e-tongue performance suggests this device as a promising routine tool for gliadin detection in foodstuffs. Finally, the gliadin quantification capability could be attributed to the polar character of the lipidic/polymeric membranes applied in the e-tongue, and also to the presence of the phosphate group in some of the membranes. Nevertheless, a wider study is required, including the validation of the methodology using different liquid and food samples. Also, a future work should include the use of nonpolar lipidic membranes in the e-tongue since they preferentially interact with glutenins, enabling the direct quantification of the gluten content, thus avoiding the controversial use of the gliadin–glutenin ratio equal to 1 to extrapolate the gluten concentration.

## ACKNOWLEDGMENTS

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## Chapter 19

# Electronic Tongues Applied to Grape and Fruit Juice Analysis

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## 19.1 INTRODUCTION

The increasing interest on a sustainable and high-quality production in agriculture and food industry has promoted the development of more automated and precise analytical systems for monitoring. Besides, optimized process control is essential to address safety rules and to maintain the commercial viability of an end product. This implies, among other aspects, a rapid assessment of the chemical and physical properties of raw materials, process streams, and end products. The industry of fruit juices is one of the most growing among foodstuff industry. Fruit juices are consumed worldwide, not only for their flavor, taste, and freshness, but also due to their beneficial health effects when consumed regularly.

Quality of fruit juice is checked throughout the production process. After extraction and concentration, some physicochemical parameters are checked like the sugar level, which is measured in degrees Brix, acidity, citrus oil level, pulp level, pulp cell integrity, color, viscosity, as well as the microbiological contamination. The final juice product is evaluated in terms of subjective qualities like flavor and texture by a sensory panel. These analyses are currently performed with conventional techniques and in some cases sophisticated instrumentation that are time consuming and require costly laboratory equipment. On the other hand, for sensory analysis, panels of trained technicians are required, which involves a considerable amount of resources, time, and money and it suffers from some drawbacks, like, for example, discrepancies due to human fatigue or stress, and clearly cannot be used for online measurements. Therefore, the implementation of analytical systems in fruit juice processing and quality control that could improve the actual instrumentation and will permit freshness evaluation, shelf life, authenticity assessment and quantitative analysis would be relevant. Multisensor systems combined with chemometric tools, also called electronic tongues, are especially suitable for this purpose (Escuder-Gilabert and Peris, 2010).

An electronic tongue (e-tongue) can be defined as an analytical instrument comprising an array of nonspecific chemical sensors with cross-sensitivity to different compounds and an appropriate chemometric tool for data processing. The sensor array produces a signal pattern that can be correlated to certain features or qualities of the sample (Vlasov et al., 2005). These systems are able to imitate the taste sense but also to perform classification and discrimination, qualitative analysis, and quantitative analysis of multiple components simultaneously. They have demonstrated their reliability and versatility in a broad range of fields, such as clinical diagnostics (Gutierrez et al., 2008b), environmental monitoring (Rudnitskaya et al., 2001), agro-food analysis (Gutierrez et al., 2008a), control of industrial processes (Winquist et al., 2005), and pharmaceutical analysis (Gutes et al., 2007), by using different types of sensors, especially electrochemical ones. However, it is in food quality and safety control where the applicability of these multisensor systems has been most extended (Baldwin et al., 2011).

Regarding commercial electronic tongues, currently there are two in the market. One is the α-Astree, manufactured by Alpha MOS, France (Alpha MOS, 2014). This device comprises a 16-position autosampler and an advanced chemometric software package containing various pattern recognition analysis modules like principal component analysis (PCA), partial least squares (PLS), discrimination function analysis (DFA), and soft independent modeling of class analogy (SIMCA). The detectors consist of an array of seven ion-selective field effect transistors (ISFETs) fabricated at the IMB-CNM (Spain) and coated with different polymer membranes. This e-tongue is mainly applied to the pharmaceutical industry to study drug formulations from the standpoint of taste. Other applications reported with the α-Astree are to foodstuff like tea, coffee, and fruit analysis described in this chapter. The other commercial e-tongue is the result of the research lead by Professor Toko from the University of Tokyo, Japan, in the nineties. The TS-5000Z taste sensing system from the company

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Intelligent Sensor Technology (INSENT), Japan, is based on an array of potentiometric sensors with lipid membranes able to recognize the basic tastes (INSENT, 2014). This equipment is also applied to food and pharmaceutical analysis.

Data treatment is a key issue to obtain a significant result from an electronic tongue (Esbensen, 2001). The signals recorded by the sensors have a high complexity due to the presence of cross-interference and matrix effect responses. Therefore, multivariate chemometric methods can be used to simplify the analysis, thus enabling the "fingerprinting" of each sample. The election of one or other method will depend on the application. Basically, there are three types of results that can be of interest: data exploration, sample classification, and multidetermination. The multivariate exploratory techniques are mainly unsupervised, given that no labels are imposed to the data matrix before the treatment. The paradigmatic example of these methods is the PCA (Correia and Ferreira, 2007). PCA reduces the dimensionality of the original data matrix, retaining the maximum amount of variability and information within the data. PCA is one of the most frequently used chemometric tools, mainly due to its very attractive features such as generation of a 2D or 3D graph that captures the largest percentage of the original variance. Other typical exploratory method is the cluster analysis (CA) used also to identify groups or clusters of similar samples. Next, the supervised statistical techniques are known for providing classification indices to a large set of samples and/or response variables (Berrueta et al., 2007). Supervised techniques include linear discriminant analysis (LDA), partial least squares-discriminant analysis (PLS-DA), *k*-nearest neighbor (*k*-NN), or SIMCA. Later algorithms are a recommended method in food-authenticity applications given their capacity of characterizing individual classes in a totally independent way and defining an enclosed class space based on a statistical confidence level. Finally, the multidetermination of continuous properties or parameters are modeled and predicted by regression methods, among which PLS regression, multiple linear regression (MLR), and principal component regression (PCR) are the most common. Although the algorithms are essentially different, these three multivariate calibration techniques produce regression models for linear systems. This means that the relationship between the parameters to determine and the variables corresponds to a linear equation. Therefore, these techniques are especially suitable for amperometric or spectrometric measurements. For nonlinear data like that from voltammetric and potentiometric sensors, there is another version of PLS called nonlinear PLS (NL-PLS) where the relationship includes a polynomial function. On the other hand, artificial neural network (ANN) is a powerful chemometric tool, suited for modeling both linear and nonlinear systems and for constructing both calibration and classification models. It is probably the most versatile tool for multivariate analysis because virtually all parameters within the architecture of an ANN can be changed, producing a large number of models for the same problem. However, this is a method more difficult to optimize in order to obtain a good calibration model (Richards et al., 2002).

In this chapter, the use of e-tongues based on chemical sensors applied to fruit juice analysis and their combination with other type of devices like e-noses is reviewed. The organization of this chapter has been done according to the type of fruit analyzed. Taking into account this criterion, this chapter includes four sections devoted to orange juice and combinations of different beverages; apple juice; other fruits like strawberry, apricot, and tomato juice; and finally grape juice.

## 19.2 ORANGE JUICE AND COMBINATIONS OF DIFFERENT BEVERAGES

The group of Professor Legin from St. Petersburg University, Russia, was one of the precursors of the e-tongue concept in Europe. This group, in collaboration with the group of Professor D'Amico from the University of Rome, Italy, developed an electronic tongue based on two types of potentiometric sensors: ion-selective electrodes (ISE) based on PVC membranes and sensors based on chalcogenide vitreous materials, with enhanced cross-sensitivities (Legin et al., 1997). An array of 18–21 sensors was applied to several beverages (soft drinks, tea, coffee, mineral water, beer) and orange juice and in particular to monitor the process of aging of orange juices during 1 week and under two temperature conditions. Using a PCA plot, three different phases of orange juice evolution with time were distinguished with a good performance: the first phase after the first 5 h of package opening, indicating a rapid evolution of juice composition; the second phase for the next hours until 122 h, indicating slow variation of composition; and the third phase at the 7th day corresponding to the deterioration of the juice. Other works using potentiometric sensors were those from the group of Professor Wróblewski from the Warsaw University of Technology, Poland. The first paper (Ciosek et al., 2004a) describes the use of an array of 16 ISEs with conventional membranes for ions (ie, $Ca^{2+}$, $NH_4^+$, $Na^+$, $Cl^-$, $HCO_3^-$) and partially selective membranes containing mixtures of ionophores and a pH electrode. Five local brands of orange juice were analyzed with PCA showing that three brands were easily distinguished from each other and the other two brands were partially overlapped (Fig. 19.1). These juices were analyzed lately with a supervised method like PLS-DA and compared with previous results with PCA, demonstrating that both methods presented similar results (Ciosek et al., 2005). A flow-through electronic tongue system based on miniaturized solid-state

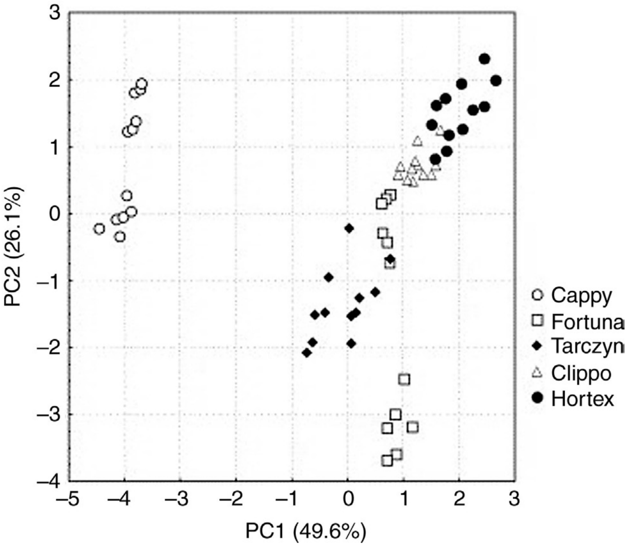

**FIGURE 19.1** **PCA scores plot for five brands of orange juice.** *(Reprinted from Ciosek et al., 2005, copyright 2005, with permission from Elsevier.)*

sensors including some chemically modified ISFETs (CHEMFETs) was developed by the same group (Ciosek et al., 2006). The miniaturization of the electronic tongue using an integrated sensor array was also described. Planar solid-state microelectrodes based on a typical printed circuit board technology, coated with different metal pastes, and modified with PVC membranes were used. Five brands of orange juice were analyzed and data treated with PLS obtaining a good classification (Ciosek et al., 2007). Here miniaturization was indicated as advantageous for the use in portable systems.

The group of Professor D'Amico reported an array of six metallic potentiometric sensors (copper, tin, iron, aluminum, brass, and stainless steel) together with a pH glass electrode. By using the nonsupervised PCA model, this system was able to distinguish the different juices tested: peach, orange, pineapple, and grape. Besides, by using the supervised SIMCA, it was also able to classify the juices according to the manufacturer (Lvova et al., 2006).

The group of Professor Del Valle from the Universitat Autònoma de Barcelona (UAB), Spain, has developed several e-tongue systems using all solid-state potentiometric sensors and PVC-based membranes. In the reference by Gallardo et al. (2005) was described a system formed by 6 ISEs able to classify 36 orange-based drinks according to the natural orange fruit content. Another e-tongue using a biosensor array of glucose oxidase enzymatic sensors containing in the membrane different amounts of metallic catalysts and using the sequential injection analysis (SIA) technique was described for quantitative analysis of glucose and ascorbic acid in fruit juice samples (Gutes et al., 2006). Using ANN for data treatment, the system demonstrated a good ability to determine glucose with a good accuracy; meanwhile the error obtained for ascorbic acid was too high. This error was attributed to the oxidation of ascorbic acid with time.

A more recent paper from Professor Machado group, from the University of Porto, Portugal, describes the use of an array of 36 all solid-state ISE with lipo/polymeric PVC membranes for the discrimination of juice soft drinks—orange, pineapple, mango, peach and strawberry, and mixtures of all them—according to the fruit juice content (Dias et al., 2011). Using LDA the authors classified four groups of juices with contents from 50 to <5% of juice with only 4 of the electrodes. Besides, quantitative analysis of fructose and glucose content of these fruit juices with PLS and MLR was performed with 16 electrodes. Reference data was obtained from high-performance liquid chromatography (HPLC), obtaining a good correlation between both methods.

One of the first works on electronic tongues was from Winquist and coworkers from the University of Linköping, Sweden. They developed voltammetric sensors and applied electronic tongues to different foodstuff (Winquist et al., 1997). According to the authors, the voltammetric technique has insufficient selectivity for specific analysis in complex media with several redox components due to the difficulties of signal discrimination. This drawback can be exploited by an e-tongue since the huge information contained in a voltammogram can be analyzed with multivariate methods. The first developed electronic tongue contained only two metal electrodes (Pt and Au). Beverages like orange and apple juice, orange still drink, and milk were analyzed. Using differential pulse voltammetry technique and PCA for data treatment, this simple e-tongue was able to markedly differentiate the orange juice from the orange still drink. The aging process of orange juices was also studied and the results indicated the capability of these systems

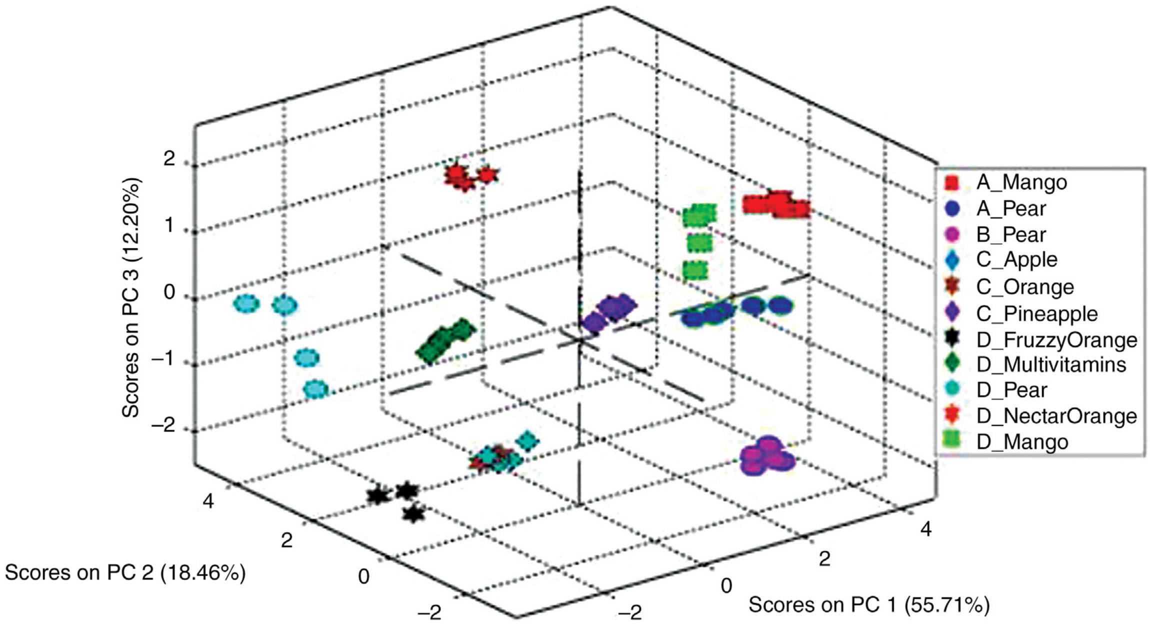

**FIGURE 19.2 Three-dimensional scores plot for 11 fruit juices with data fusion from e-nose and e-tongue.** *(Reprinted from Haddi et al., 2014, copyright 2014, with permission from Elsevier.)*

to distinguish between the first hours of aging, related to the evaporation of volatile compounds and aged samples due to oxidation processes (ie, ascorbic acid oxidation). This system was later improved with four working electrodes of gold, iridium, platinum, and rhodium and applied to different fruit—kiwi, orange, peaches—and tomato juice classifications (Holmin et al., 2001). In this work the large data sets obtained with the voltammetric scans were treated with compression methods like wavelet transformation (WT) and hierarchical PCA (HPCA). The results demonstrated that the samples were clearly separated by the type of fruit and even Dutch and Spanish origin of tomato fruit was distinguished. Another voltammetric e-tongue using Pt, Au, and poly(3,4-ethylenedioxythiophene) (PEDOT)-modified electrodes was described lately for orange juice classification among other fruit juices—orange, pear, peach, and apricot juices—and differentiation of orange juice brands. It was demonstrated that the PEDOT electrode had superior discriminant ability than the bare metallic electrodes for differentiating between fruit juice types and also brands of orange juice (Martina et al., 2007). However, after each sample measurement, the membrane of this electrode was degraded and had to be changed.

As reported, the majority of groups working on e-tongues are using the same type of sensors. A few works have been described regarding the use of arrays of different sensors (ie, optical and electrochemical) or even the combination of an e-nose and an e-tongue increasing the potentiality of these devices. The group of Gardner from the United Kingdom developed a combined e-nose and e-tongue based on surface acoustic wave (SAW) sensors for liquids and CHEMFETs for gases. These later sensors are modified with conducting polymeric membranes containing carbon nanospheres. Here the most significant result was the discrimination of samples of orange juice from those of milk and water using the combination of gas and liquid sensors (Cole et al., 2011). The fusion of data from an e-tongue based on six PVC-membrane potentiometric sensors, an e-nose based on five tin oxide-based gas sensors (Toguschi gas sensors), and a humidity sensor has recently been described (Haddi et al., 2014). In this application, the set of samples used had a high variability: a total of 46 samples of fruit juice (pear, apple, orange, mango, pineapple, multivitamin, etc.) from 4 different brands and with different percentages of fruit content. These samples were measured and treated with PCA. According to the results, the e-tongue was able to discriminate among the different fruit juices, but the fusion with the e-nose improved notably the results. In Fig. 19.2, the 3D PCA plot for this fusion approach is shown. As can be seen, this representation explains the 86.37% of the total variance and the eleven fruit juices from different manufacturers (A, B, C, and D) were well distinguished. Only the samples from C_Apple and C_Orange were overlapped.

The Astree e-tongue from Alpha MOS has been also applied to orange juice analysis. As reported in Baldwin et al. (2011), the e-tongue was able to separate between juices from fruit harvested from healthy trees and those harvested from Huanglongbing (HLB)-infected trees, which were symptomatic for the disease (small, green, and lopsided fruit) or asymptomatic (normal-looking fruit). These results were comparable with those from a trained sensory panel.

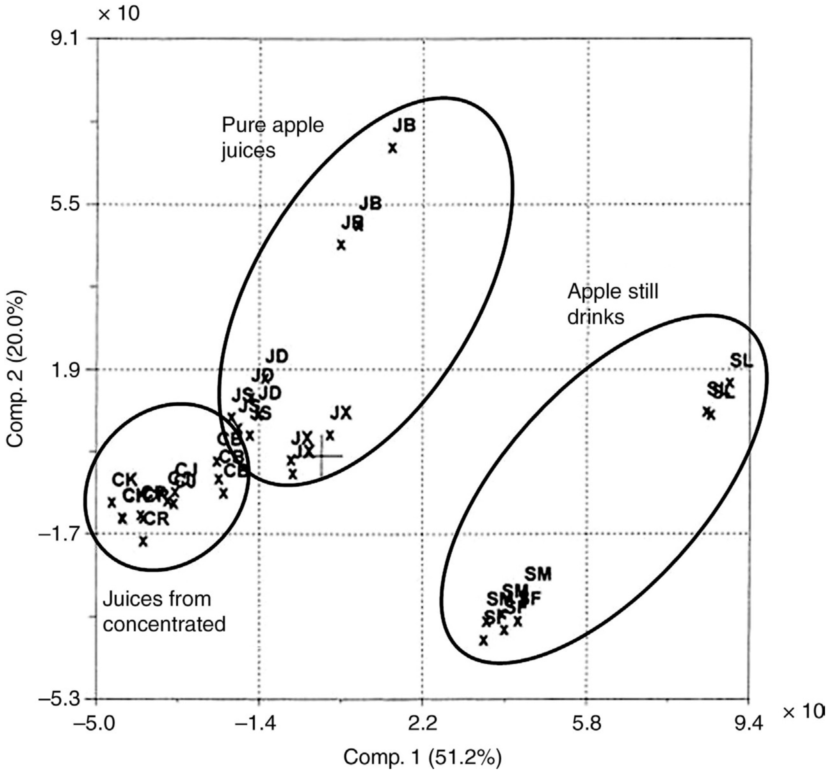

**FIGURE 19.3** **A PCA score plot for different samples of apple juices.** *JB*, *JD*, *JX*, and *JS* are different pure juices; *CB*, *CJ*, *CK*, and *CR* are samples from concentrated juice and diluted with distilled water; and *SL*, *SM*, and *SF* are different apple still drinks. *(Adapted from Winquist et al., 2002, copyright 2002, with permission from Elsevier.)*

This instrument, combined with an e-nose from Applied Sensor, Sweden, was also used to study the differences between treatments of various processes for fruit preservation (Hartyáni et al., 2011). Two treatment methods were tested: pulsed electric field (PEF) and high hydrostatic pressure (HHP) and compared with typical heat treatment for preservation. Results demonstrated that there were differences between fruit juices treated with these two methods. The Astree e-tongue was also applied to the analysis of six brands of orange beverages containing a low percentage of fruit juice (Liu et al., 2012). This paper described the use of several chemometric tools like PCA, CA, and SIMCA and established the optimal number of sensors for classifying the samples according to the five basic taste descriptors. The results demonstrated that PCA and CA models permitted the differentiation of two brands; meanwhile the other brands were not clearly separated.

## 19.3 APPLE JUICE

The first paper related to the application of electronic tongues to apple juice analysis was that from Winquist et al. (1997), described previously. An evolution of this first concept was presented by the same group in 2002 incorporating a third working electrode, based on rhodium, and combined with a flow injection analysis (FIA) system (Winquist et al., 2002). By using the PCA technique, it was possible to discriminate between three different juice classes: pure juice, juice that was made from concentrated, and apple still drinks as shown in Fig. 19.3.

A potentiometric sensor array was also developed to analyze apple juices. Concretely, the responses of ISEs based on PVC membranes with different ionic selectivity were used to construct a PCA model able to classify 10 different brands of apple juice (Ciosek et al., 2004b). However, the more significant result of this work was the description of a new statistical procedure for reducing the number of sensors in the array. It is well known that one of the most important steps in a multivariable analysis is the election of the variables (sensors) that will generate the best model. Once the classification is performed, the methodology consists on quantifying the ability of each individual sensor to discriminate between different classes of samples. The idea is to obtain the maximum information of the sample, but with the minimum number of sensors in order to simplify the experimental setup and the model management, as well as to reduce redundant and unsubstantial information. In this case, the original 16-ISEs array was reduced to 9-ISEs, providing similar or even better discrimination of juice samples.

The Astree electronic tongue was also tested for apple juices. Results from this device combined with the electronic nose Prometheus, also from Alpha MOS, were correlated

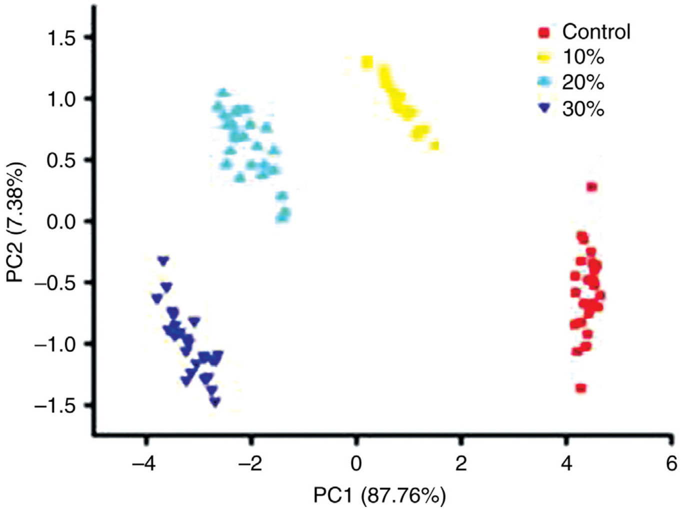

**FIGURE 19.4 PCA plot for four tomato juice groups unadulterated and three adulterated groups: 10, 20, and 30% of overripe tomato based on ANOVA selected fusion dataset.** *(Reprinted from Hong et al., 2014, copyright 2014, with permission from Elsevier.)*

to the human sensory assessments and consumer acceptance. The results demonstrate that the hybrid combination of electrochemical and gas sensors can be a useful tool to predict up to 32 attributes used to describe the taste of apple juices (Bleibaum et al., 2002). Kovacs and coworkers correlated the measurements of the instrument with some taste attributes, like "apple taste," "sweet taste," or "sour taste." Not only the PCA model was able to follow the tendency obtained by the sensory evaluation, but also a good PLS prediction model was obtained for the quantification of "apple taste" (Kovacs et al., 2011). The Astree e-tongue was also used to investigate the effect of high-power ultrasound and pasteurization on the characteristics of pure (100%) apple juice and 50% apple nectar. These two methods of preserving fruit juices produce changes in the chemical composition that the Astree e- tongue was able to detect and to differentiate from the untreated samples (Simunek et al., 2013).

## 19.4 OTHER FRUIT JUICES

There are several applications to tomato juice using data fusion of commercial equipments, concretely the Astree e-tongue and the PEN2 e-nose (Airsense Analytics, Schwerin, Germany), which is composed of 10 metal oxide semiconductors (MOS) of different chemical composition and thickness to provide selectivity toward volatile compounds (Hong et al., 2014). The most interesting results in this study were the use of adulterated samples with 10, 20, and 30% w/w of overripe tomato juice. By using analysis of variance (ANOVA) of selected variables and PCA, the fusion approach presented the best results, as can be observed in Fig. 19.4. The three groups of adultered samples and the control were perfectly differentiated. Besides, the results proved that it is possible to measure the freshness of fruits used for juice elaboration.

The Astree system was also used to analyze apricot juices from three Hungarian varieties: Gönczi, Ceglédi, and Pannónia. The results demonstrated that the e-tongue is a promising tool for monitoring the effects of postharvest techniques on the fruit-ripening process (controlled atmosphere storage or the treatment with 1-methylcyclopropene as a preservative). Besides, the classification of apricot varieties using DA and the determination of correlation between e-tongue, chemical properties, and sensory analysis were successful (Kantor et al., 2008). Also the Astree e-tongue was applied to strawberry juice analysis, but in this case in combination with the PEN2 E-nose (Qiu et al., 2014). This work presented an interesting study with five types of strawberry juices based on different processing approaches for preservation (microwave pasteurization, steam blanching, high-temperature short-time pasteurization, frozen-thawed, and freshly squeezed). In conclusion, the Astree system reached a higher accuracy rate compared to PEN2, both in the qualitative analysis (classification of the juices) and in the quantitative analysis [multidetermination of these parameters of quality control: vitamin C, pH, total soluble solid (TSS), total acid (TA), and TSS/TA ratio]. The data fusion of the two systems offered a slight advantage in the LDA classification and PLS regression.

## 19.5 GRAPE JUICES

The characterization and identification of the juice obtained from *Vitis vinifera* grapes (addressed to consumption) is a necessary task in viticulture. The grape quality control permits the enologists to decide the procedure of wine elaboration. For example, the control of grapes' ripeness by the sugar content, defined as the degrees Brix, as well as the probable volumetric alcoholic degree (VAD), is of vital interest to decide the date of the grape harvest. Moreover, the

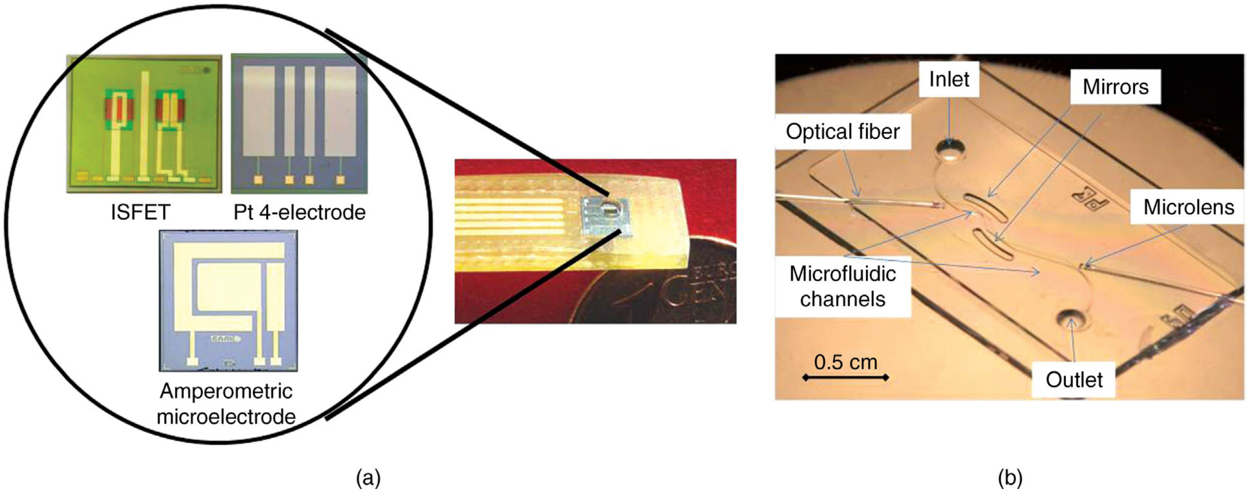

**FIGURE 19.5 Picture of the microsensors used.** (a) Chips corresponding to electrochemical sensors and (b) optical MIR system. *(Reprinted from Gutierrez-Capitan et al., 2013, copyright 2013, with permission from American Chemical Society.)*

total acidity of the grape juice allows calculating the ripening index of the fruit. On the other hand, the complexity of the grape juice is significant as a sample, given its rapid changes in the chemical composition (sugars, ethanol, pH, amino acids), physical properties (turbidity, density, color), and varietal aromas. For that reason, grape juice requires rapid and reliable measurements in order to actuate in an efficient way during the elaboration process. Nowadays, the characterization and authenticity of grape juices is carried out with the analysis of the protein fingerprint or the residual DNA (Le Bourse et al., 2010). These methods are time consuming and require complex and expensive equipment. For this reason, the use of e-tongues would be advantageous.

The first paper describing an e-tongue system applied to grape juice analysis was reported by the group of Jimenez-Jorquera from IMB-CNM, Spain (Moreno i Codinachs et al., 2008). The multisensor e-tongue system described was based on integrated arrays of ISFET sensors and interdigitated electrode (IDE) structures (Codinachs et al., 2008). Six ISFET sensors were modified with polymeric membranes sensitive to ions $Na^+$, $K^+$, $Ca^{2+}$, $NH_4^+$, and $Cl^-$ and other six with chalcogenide glass membranes sensitive to heavy metal ions ($Pb^{2+}$, $Cd^{2+}$, $Cu^{2+}$, $Tl^+$, $Ag^+$). Both multisensor chips contained also a pH ISFET and they were implemented in a FIA system. By using a PCA model, the samples showed a clear clustering for the four grape varieties analyzed (Airen, Macabeu, Malvasia, and Chardonnay) and by using the PLS technique, it was possible to determine the degrees Brix, the probable VAD, pH, and total acidity with a relative error below 10% for all the predictions.

Last e-tongue was improved including other types of sensors in order to obtain the maximum information about the grape juices. This hybrid e-tongue was constituted by seven ISFET potentiometric sensors sensitive to pH, common ions and generic ones, a conductivity sensor, a redox potential (ORP) sensor (both with a Pt 4-electrode configuration), and two amperometric electrodes: a gold (Au) microelectrode and a microelectrode for sensing electrochemical oxygen demand (EOD). Besides, an optofluidic system consisting on a multiple internal reflection (MIR) system fabricated by soft lithography was incorporated in order to obtain information about the color of the sample. Photographs of the chips used, the probe with a chip encapsulated, and the MIR system are shown in Fig. 19.5. This array of sensors was applied to the classification and characterization of white grape juices from three reference varieties (Albariño, Muscat, and Palomino) using the PCA technique. The distribution of these samples in a 2D plot, which explained 59% of variability, is shown in Fig. 19.6a. These three genotypes were selected as reference because their grape juices present extreme or intermediate characteristics. For example, Muscat à Petit Grains Blanc has a high aromatic intensity, while Palomino produces a neutral juice with low acidity, and Albariño presents intermediate juice characteristics between the other two grapes and a high acidity. A more detailed study of the PCA model for the loading plot demonstrated that the PC 1, which distinguished Albariño, is constituted basically by the pH ISFET and a set of variables related with the oxidoreduction properties. On the other hand, the optical and ionic variables had more importance in the PC 2 loading plot, which separated Muscat from Palomino. The same variables were used to construct a SIMCA model in order to study in more detail the feasibility of the hybrid electronic tongue to distinguish between different grape juices. Once the model for the three reference varieties was performed, a set of 22 new juices were interpolated. In Fig. 19.6b, the Coomans diagram for the classification of the Albariño and Muscat models is depicted. The prediction values are shown with a probability of 95%. In the *x*-axis of Fig. 19.6b, the distance to the Albariño model is shown. Grape juices that

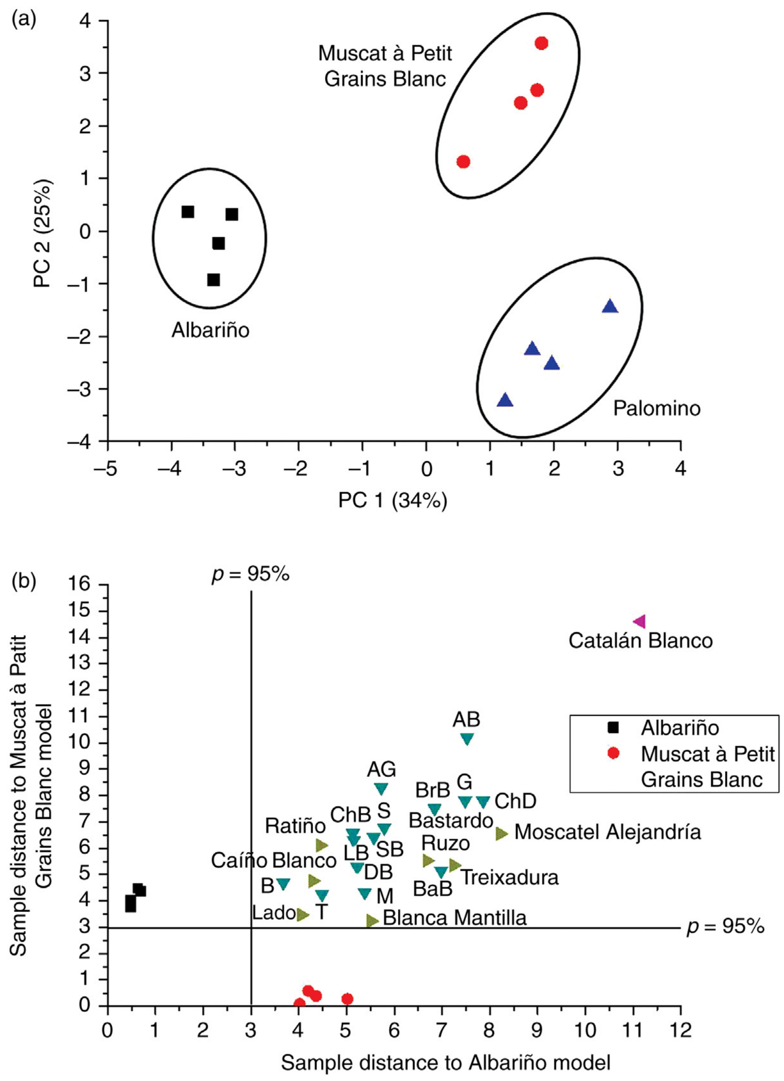

**FIGURE 19.6** (a) PCA results for the grape juices reference samples of Albariño, Muscat à Petit Grains Blanc, and Palomino. (b) Coomans diagrams for the classification in the Albariño and Muscat models. *(Reprinted from Gutierrez-Capitan et al., 2013, copyright 2013, with permission from American Chemical Society.)*

are placed between 0 and 3 on the *x*-axis belong to Albariño. In the *y*-axis, the distance of the samples to the Muscat model is shown. Hence, grape juices that are placed between 0 and 3 on the *y*-axis belong to Muscat. Samples that are placed in the area corresponding to $x, y > 3$ do not belong to any of these models. On the other hand, juices that are located in the area $x, y < 3$ of the plot belong to both models. As can be observed, the results demonstrated that the Albariño and Muscat juices were located inside their respective models and well distinguished from the other grape varieties (Gutierrez-Capitan et al., 2013). Here the variety Catalan Blanco is perfectly separated from the others. This variety comes from a hybrid genotype; therefore, its characteristics are clearly differentiated from the other grape genotypes.

A new e-tongue containing biosensors was reported in Medina-Plaza et al. (2014). The array was formed by five carbon paste electrodes modified with different metalophthalocyanines as electron mediators. Besides, in the surface of these 5 electrodes, the enzymes tyrosinase and glucose oxidase were immobilized, obtaining a set of 15 different combinations that provide both global and specific information of the sample. By using a PCA model, the e-tongue system was capable of distinguishing grapes of five different varieties harvested in 2012 in the Ribera de Duero appellation according to the sugar and polyphenolic content.

## 19.6 CONCLUSION

The application of e-tongues to fruit juice analysis has been described extensively by the majority of groups working in this field. Different E-tongues based on sensors have demonstrated their capability to perform several accomplishments like discrimination of fruit juices among other types of beverages and among several fruit types; differentiation of commercial brands; assessment of aging for open packages of fruit juice; detection of presence of adulterant compounds (ie, addition of ripe fruit); detection of percentage of fruit content in still beverages; and fruit cultivar type classification and grape variety. Besides, these devices are able to classify fruit juices according to taste descriptors defined by an expert panel. E-tongues are also able to perform quantitative analysis of parameters like ascorbic acid, glucose, fructose, citric acid, and the parameters related to quality of grapes, which are of critical importance to assess the quality of juices and the wine elaboration.

Regarding the use of analytical devices, the potentiometric and voltamperometric sensors have been the most used. These sensors have been combined with other kinds of sensors like conductivity and optical sensors or e-noses. These later devices have contributed to extend the analysis and exploration of samples according to their volatile content and aromatic attributes.

The application of hybrid e-tongues to fruit analysis has evidenced the interest on using different kinds of sensors. This fact is especially remarkable for grape juice analysis. Here the use of microsensors fabricated with microelectronic technology allows both qualitative analysis and quantitative analysis with good correlation with standard techniques. The use of miniaturized sensors introduces also the option to miniaturize the e-tongue and consequently the portability of the system and the capability to perform field analysis.

It is remarkable the work described by several groups using the commercial Astree e-tongue to study different fruit juices' applications, above all those addressed to classify the fruit juice organoleptic descriptors and the correlation with sensory panels. The feasibility of this device demonstrates the high performance of the sensor technology used, based on ISFET sensors, combined with a customer software developed by the company Alpha MOS.

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Chapter 20

# Meat and Fish Spoilage Measured by Electronic Tongues

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## 20.1 INTRODUCTION

Two techniques for determining freshness (and, therefore, spoilage) of meat and fish have been traditionally used. One is a sensory test conducted by a panel of experts who control the various organoleptic attributes of the corresponding sample. The other involves determining the concentration of certain chemical or biochemical bioanalytes known to correlate with food spoilage.

### 20.1.1 Sensory Analysis

A sensory analysis was the first method used by humans for assessing freshness, and it is still one of the most important methods applied in the fish and meat sector. Properly performed sensory methods are a rapid accurate tool that provides unique food information (Martinsdóttir et al., 2001).

### 20.1.2 Analytical Techniques

Among available analytical techniques, measuring bacterial counts is one of the most commonly used procedures for determining fish and meat freshness (Kaneki et al., 2004). Measuring volatile amines from decarboxylation of amino acids is also an indicator of freshness (Vida-Carou et al., 1990). Regarding biochemical procedures, determination of concentrations of adenosine triphosphate (ATP) and some ATP degradation products, such as adenosine diphosphate (ADP), inosinic acid (IMP), inosine (Ino), and hypoxanthine (Hx), has been widely used as a relatively simple method to monitor fish and meat spoilage. The evolution of these nucleotides and the combination of their concentrations in the so-called *K*-value [Eq. (20.1)] (Saito et al., 1959) have been found to be closely related with postmortem time.

$$K(\%) = \frac{(\text{Ino} + \text{Hx})}{(\text{ATP} + \text{ADP} + \text{AMP} + \text{IMP} + \text{Ino} + \text{Hx})}100 \qquad (20.1)$$

However, these traditional techniques (ie, sensory tests) and chemical or biochemical determinations of the concentration of target bioindicators, are slow and time consuming, and need trained personnel or relatively sophisticated tools. This somewhat limits the application of these procedures in industry as they are not suitable for rapid monitoring, are relatively expensive and time consuming, and are generally for in-laboratory use only. One major effect is that these methods cannot evaluate correct meat freshness when sold on the market. In fact, the development of simple, undemanding, and nondestructive new analytical tools, which would also be low cost and could be applied to a wide range of situations for monitoring food freshness, is still an unresolved goal.

Based on these concepts, several attempts have been made to use techniques to assess fish or meat freshness in a simple, undemanding way. Some of these techniques are:

- *Image analysis*. Image processing systems play an increasingly important role in food quality evaluations as they maintain accuracy and consistency while eliminating the subjectivity of manual inspections. Image techniques, for instance, based on short-wavelength near-infrared (SW-NIR) spectroscopy have been used to predict the freshness of chicken breasts (Grau et al., 2011). Surface inspection by image analysis has also been used for monitoring the quality of fish samples (Kroeger, 2003).
- *Chromogenic sensor*. Arrays based on several chromogenic indicators capable of changing color due to a reaction with volatile compounds produced on packed fish or meat samples have also been reported as an indication of meat (Salinas et al., 2014) and fish freshness (Zaragozá et al., 2012).
- *Biosensors*. Analytical devices based on biological recognition elements (such as an enzymes, antibodies, etc.) coupled to a chemical or physical transducer. They have also been used for meat or fish monitoring. For instance,

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Hernández-Cázares et al. (2010) used an enzymatic sensor with immobilized diamine oxidase to determine the content of total amines in dry-fermented sausages. Biosensors have also been used for determining fish freshness (Frébort et al., 2000).

- *Texture measurements*. Texture has also been found to change with the evolution of meat and fish freshness (Macagnano et al., 2005). In most cases, measurements of firmness and the parameters extracted from the stress relaxation curves correlate well with the sensory attributes and dehydration or water loss.

## 20.2 ELECTRONIC TONGUES

As a complementary procedure to these methods, the electronic tongue (e-tongue) concept emerges for meat and fish freshness and/or spoilage assessment. This is a tool that has been recently introduced, and was inspired by the mode in which mammals recognize food through senses of taste. Use of e-tongues is especially appealing when characterizing complex attributes of the whole sample is an important issue. E-tongues do not use specific, but nonspecific, sensors. These are nevertheless able to respond differentially to a group of related chemical species, whose global response can relate with certain parameters or characteristics. Nonspecific sensors are usually integrated into an array, and their response is commonly analyzed by suitable pattern recognition algorithms. Note that e-noses have also been developed for fish and meat spoilage assessments (Musatov et al., 2009; El Barbri et al., 2007), but are not reviewed herein.

An easy way to build up an e-tongue is to employ a set of electrodes and follow potentiometric or voltammetric electrochemical techniques. In fact, several electronic tongues based on electrochemical sensors have been developed. Among them, those which rely on potentiometric measurements have been widely used by employing, for instance, ion-selective electrodes. One alternative to avoid employing membrane-containing sensors is to use simple metallic wires as suitable electrodes in e-tongue devices. E-tongues with metallic electrodes are very simple to prepare and easy to use. Responses with these electrodes in the potentiometric mode are based on the spontaneous polarization of metals and other elements in the presence of certain chemical species. Simple metallic electrodes have also been used to design voltammetric e-tongues.

E-tongues were initially developed for measurements in liquid samples where contact between electrodes and sample is no problem. In particular, the first applications in foods focused on the qualitative analysis of beverages, such as wine, juice, milk, and water (Escuder-Gilabert and Peris, 2010). However, increasing interest has been recently shown in the potential use of e-tongues in solid samples, such as meat and fish. In particular, one field of interest is monitoring meat and fish spoilage, typically under refrigeration. These food items are solid, but have a high water content. Therefore, they are perishable and have a very limited useful commercial life. Presence of water also makes the use of e-tongues suitable in these samples, where close contact between electrodes and samples normally needs to be achieved.

As stated previously, the response of the electrodes used in e-tongues is quite nonspecific. Thus, to draw conclusions, the employment of multivariate analysis techniques, such as a principal component analysis (PCA), is necessary. Many studies that report the application of e-tongues for fish and meat spoilage have attempted to correlate the electrochemical data obtained from the tongue with certain physical, chemical, or biological parameters that are commonly used to assess meat or fish spoilage. In such cases, supervised techniques [eg, partial least square (PLS)] or artificial neural networks [ANN, eg, multilayer perceptron (MLP-ANN) and Fuzzy Artmap] tend to be used.

Later we will provide details of the use of potentiometric and voltammetric e-tongues in studies into meat and fish spoilage. Some examples of the utilization of impedance spectroscopy, in which a multivariate analysis was applied to obtain a correlation between electronic data and spoilage, are included.

## 20.3 MEAT AND FISH SPOILAGE ASSESSMENT USING E-TONGUES

This section provides a description of the most significant contributions made in the e-tongue field for determining freshness and spoilage of meat and fish. The narrative is provided according to the electrochemical technique used: potentiometry, voltammetry, and impedance spectroscopy.

### 20.3.1 Potentiometric E-Tongues

The potentiometric electrochemical technique measures the potential generated spontaneously between an active electrode and a reference electrode.

#### *20.3.1.1 Meat Spoilage Studies*

One of the first works to have employed a potentiometric e-tongue to determine meat freshness was done using an array of nonspecific electrodes of different materials (ie, Pt, CuS, and $Ag_2S$) (Kaneki et al., 2004). The authors took measurements with these electrodes on pork meat samples over 6 days. Then they related these data with certain chemical and biochemical determinations using PCA and multiple regression analysis (MRA) techniques. They observed that the potential of the electrodes diminished according to not only the formation of putrescine and dimethyl sulfide, but also to the increase in the bacterial counts produced by meat putrefaction.

Some of us have conducted several studies on pork freshness analyses using an e-tongue based on a family of simple metallic electrodes. It is known that metal electrodes produce a different potential according to the nature of both the metal and the sample, and their response has been reported to be related to the redox potential (Soto et al., 2006). For this specific application in meat spoilage studies, metallic electrodes were used as rods (0.8 mm in diameter), which were easily inserted into meat tissue to establish a close contact between the electrodes and the sample. Gil et al. (2010) employed a set of six electrodes (Au, Ag, Cu, Pb, Zn, and C), which were applied to monitor pork freshness under refrigeration over 12 days. At the same time, pork meat spoilage was followed by the determination of certain chemical and biochemical parameters: pH, microbial count, and concentrations of IMP, Ino, and Hx. Data were studied by artificial neural networks: MLP-ANN and Fuzzy-Artmap. A correlation between these parameters and the e-tongue data was achieved by a PLS analysis (Gil et al., 2011).

#### *20.3.1.2 Fish Spoilage Studies*

A similar e-tongue, also with rod-shaped metallic electrodes, was used to monitor fish freshness. Barat et al. (2008) first demonstrated the good correlation between aqueous solutions with a different pH, redox potential and cysteine concentrations, and the electric potential of silver and gold electrodes. Based on these studies, the authors extended their work to crushed and whole sea bream samples. Gil et al. (2007) demonstrated that the e-tongue was able to classify a certain fish sample as being apt for consumption, doubtful, and non apt by MLP-ANN (Fig. 20.1) and Fuzzy Artmap networks.

In another work, potentiometric measurements were correlated with biochemical analyses done on sea bream using a similar e-tongue. In particular, changes in the concentration of ATP-related compounds IMP, Ino, and Hx (determined by HPLC), versus the postmortem period for sea bream samples were studied. Gil et al. (2008b) found that the potentiometric data measured with the e-tongue correlated well with the *K*-value [Eq. (20.1)].

In addition to rod-shaped electrodes, the same authors tested the potential use of flat electrodes on sea bream. Electrodes were built using screen printing thick-film techniques from pastes with different active elements (Martínez-Máñez et al., 2005). A good relationship was observed between the responses of electrodes with various physicochemical parameters related with fish spoilage, including a correlation with the concentration of several biogenic amines, such as histamine, tyramine, and cadaverine (Gil et al., 2008a).

Heising et al., focused on using an ion-selective electrode for ammonium and its potential application as a freshness indicator in cod (*Gadus morhua*) samples. The results correlated with changes in total volatile basic nitrogen (TVB-N) values and tetramethylammonium concentrations (Heising et al., 2012).

Zhang et al. (2012) utilized a commercially available e-tongue (Alpha Mos) and MALDI-TOF/TOF MS/MS studies to identify the peptides responsible for puffer fish taste (*Takifugu obscurus*). The same commercial e-tongue was used to study spoilage of *Parabramis pekinensis*, and measurements were taken at the same time to detect TVB-N and total viable counts (TVC) (Han et al., 2014a). A good relation between chemical and e-tongue data was observed by PLS and support vector regression (SVR). Han et al. (2014b) combined the same e-tongue with an electronic nose (based on nine metal oxide semiconductor gas sensors) and ran a chemometric analysis for qualitative fish freshness discrimination.

## 20.3.2 Voltammetric E-Tongues

Another widely used electrochemical method in e-tongues is voltammetry. In this technique, a specific varying electric potential is applied to a set of electrodes with a three-wire potentiostat system. Then the electrical current flowing

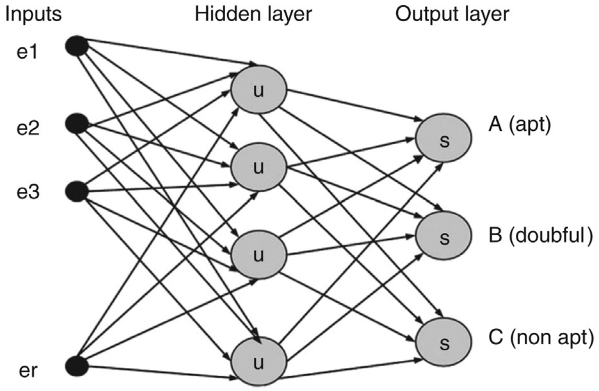


**FIGURE 20.1** **Perceptron artificial neural network outputs for three possible states of fish for consumption.**

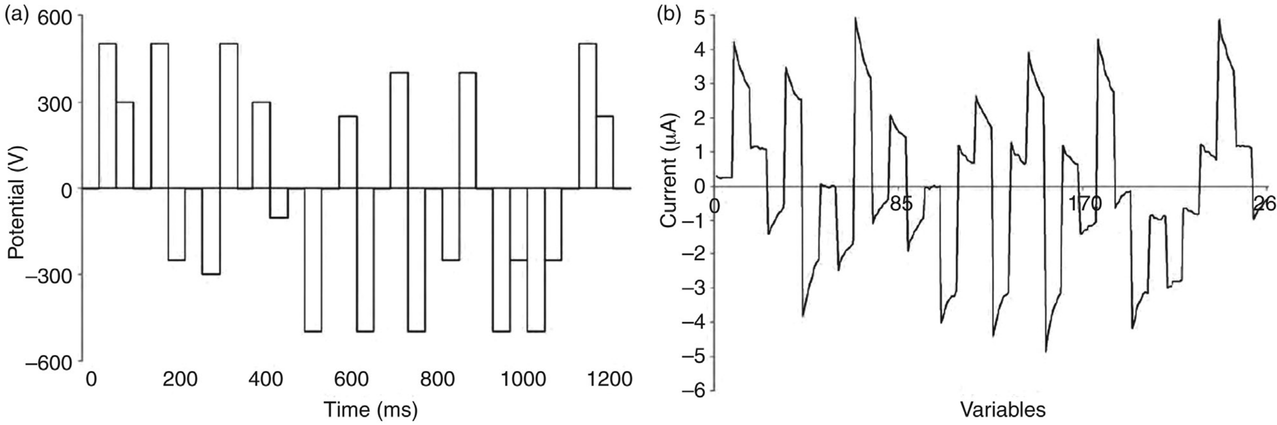


**FIGURE 20.2** (a) The applied potentials and (b) the current response of Variable Amplitude Pulse Voltammetry (VAPV).

through the electrodes is monitored. The measured current is proportional to the applied potential and the presence of both electroactive and nonelectroactive species in the sample. A number of parameters (number of pulse, amplitude of pulse, etc.) can easily be selected by this technique. Basically, two types of voltammetry are used in e-tongues: cyclic and pulse voltammetry. Fig. 20.2a shows, by way of example, a typical set of pulses applied to the sample. Fig. 20.2b displays the corresponding electrical current flowing through a certain electrode. This electrochemical response is a fingerprint that correlates with the presence of certain electroactive and nonelectroactive species in solution.

### *20.3.2.1 Meat Spoilage Studies*

The application of voltammetry-based e-tongues to meat pieces is difficult because the electrode assembly usually has a flat surface, which hinders close contact being made with the meat sample. So it not surprising that there are very few examples in this area. In this context, Noh et al. (2011) used cyclic voltammetry as a suitable method to determine beef loin freshness during cold storage using an interdigitated electrode composed of gold electrodes printed on a silicon wafer. They found that the shape of the cyclic voltammogram changed from a roundish curve to a shapely curve with longer storage periods.

### *20.3.2.2 Fish Spoilage Studies*

The application of voltammetric e-tongues in studies on fish for spoilage determination is relatively recent. In this field, Rodríguez-Méndez et al. (2009) performed a seminal study which attempted to correlate the response given by an array of voltammetric sensors with fish freshness. They used an array of screen-printed electrodes (SPE) and another array formed by classic carbon paste electrodes (CPE). The arrays of voltammetric sensors were chemically modified with phthalocyanines. The sensors showed good sensitivity to model solutions of biogenic amines (ie, ammonia, dimethylamine, trimethylamine, cadaverine, and histamine), which considerably influenced the electrochemical behavior of electrodes. The pattern of responses given by the array was successfully applied to evaluate fish freshness and to determine the postmortem period. The study showed that the signals provided by classical CPE were better resolved and displayed better sensor-to-sensor reproducibility than SPE. The same research group extended their study of fish freshness monitoring by introducing multisensory systems based on carbon screen printed electrodes modified with polypyrrole and doped with different doping agents (Apetrei et al., 2013). The system was applied to monitor fish freshness of pontic shad (*Alosa pontica*). An increase in the signal currents associated with biogenic amines was observed with more storage days.

A homemade voltammetric e-tongue (Fig. 20.3), formed by four noble (Au, Pt, Ir, Rh) and four nonnoble (Ag, Cu, Ni, Co) metallic electrodes (Alcañiz et al., 2012), was applied to monitor fresh cod (*G. morhua*) spoilage, and also in a shelf-life assessment in cold storage (Ruiz-Rico et al., 2013). For this purpose, physicochemical and microbial analyses were carried out, and measurements were also taken with the e-tongue. The voltammetric tongue was able to discriminate between fresh and spoiled fish. The statistical models obtained with the e-tongue measurements successfully predicted certain physicochemical and microbial parameters, such as TVB-N and mesophilic bacteria counts.

## 20.3.3 Electrochemical Impedance Spectroscopy

Electrochemical impedance spectroscopy studies a system's response to the application of a small amplitude alternate current signal with different frequencies. Customarily, the user selects both frequencies (normally between

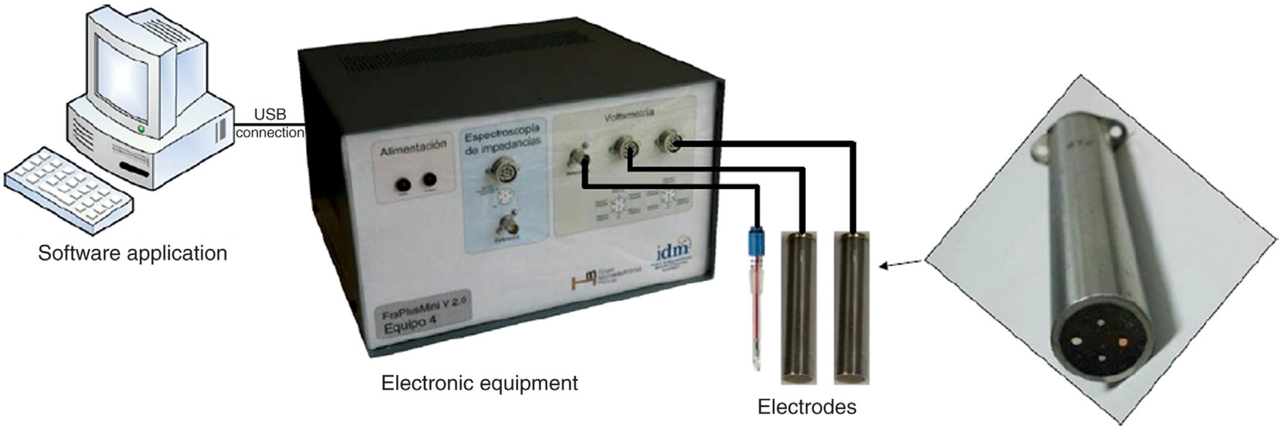


**FIGURE 20.3 Electrodes and voltammetry equipment built at IDM of UPV.**

1 and 1 MHz) and the amplitude of sinusoidal voltage signals. For each frequency, the electronic equipment generates the corresponding sinusoidal voltage waveform, which is applied to the electrode. The current and voltage signals at the electrode are then sampled and the collected data are sent to the computer, where a Fourier analysis is performed to determine their amplitude and phase. From these data, the module and phase of the sample's equivalent impedance for the current frequency are calculated. The obtained data can be plotted on two graphs, the module plot and the phase plot, where the values of the impedance module and phase versus frequency are plotted (Fig. 20.4). The results are usually discussed according to the study done of the shape of the modulus and phase graphs. Another approach consists in considering the value of each module and phase as vector data to perform a multivariate analysis. In this case, although only one electrode is usually used, data are analyzed as if the number of electrodes were the same as the frequencies used.

### 20.3.3.1 Meat Spoilage Studies

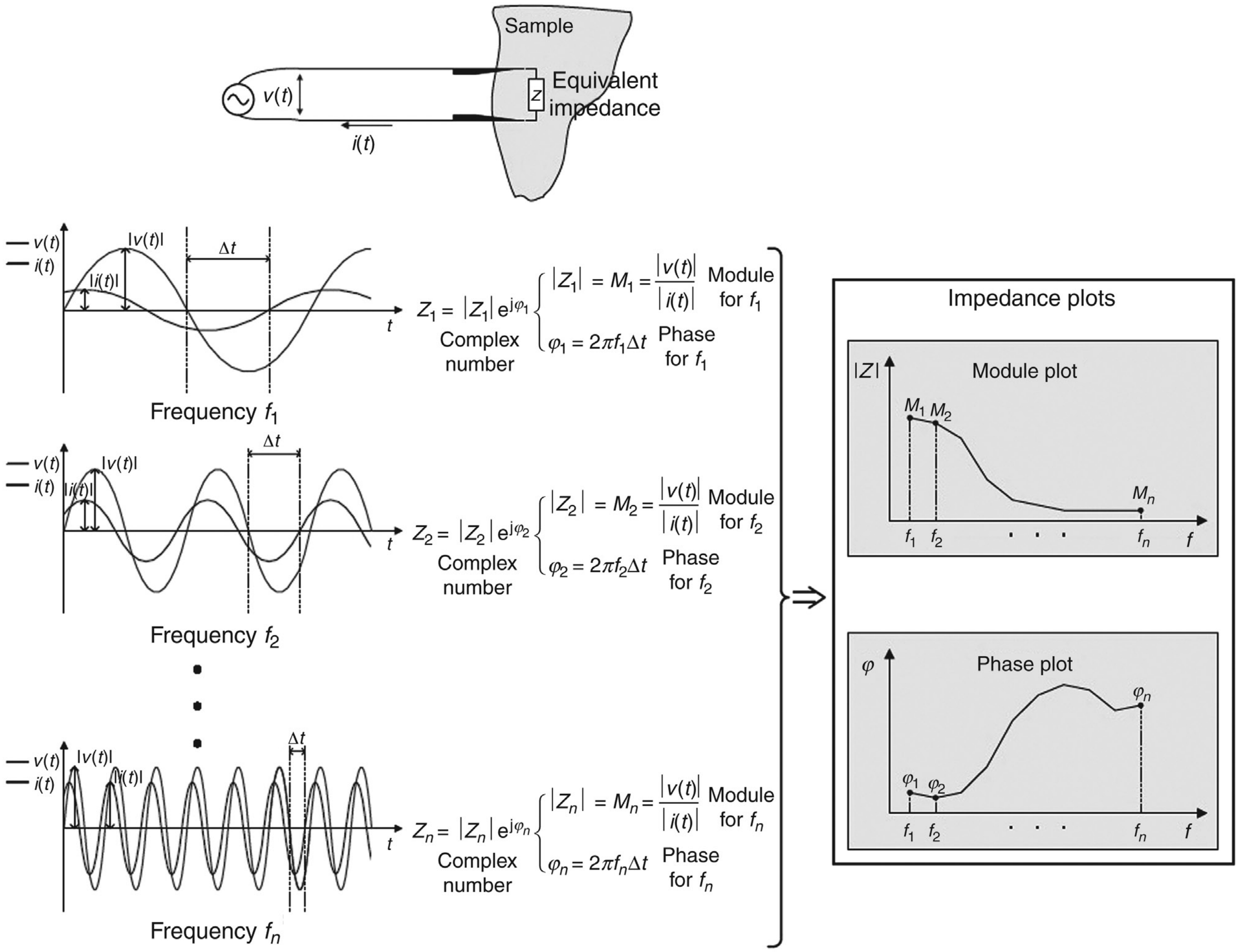
Impedance spectroscopy has been widely used to determine meat spoilage. Salvat et al. (1997) compared the impedance technique with a standard plating method to detect aerobic bacteria (*Pseudomonas* ssp.) associated with poultry meat decay. Impedance spectroscopy has been applied by Damez et al. (2008) to study the electrical anisotropy behavior of beef meat during maturation to early assessment of meat aging. In particular, these authors worked according to the concept that a simple measurement of electrical and dielectric parameters can be linked to meat fiber strength. Dielectric properties have also been used by Castro-Giráldez et al. to estimate the spoilage progress of pork meat. A PCA has been employed to describe relations between meat's physical/biochemical parameters and dielectric parameters (Castro-Giráldez et al., 2011). They obtained good correlations between dielectric properties and some texture-related aging parameters, such as hardness and chewiness.

A portable system for measuring impedance spectroscopy, capable of working with 50 frequencies between 1 and 1 MHz, has been recently developed by some of us (Masot et al., 2010). This equipment incorporated two types of electrodes: a coaxial needle (Fig. 20.5), used to determine salt content in different samples (Garcia-Breijo et al., 2008); a double electrode (Fig. 20.5). The device was employed to analyze the quality of whole pieces of pork ham (De Jesús et al., 2014). Impedance data were able to discriminate between altered and unaltered dry-cured hams. A tendency to classify between deep spoilage and swollen hams was also shown by the authors.

Impedance spectroscopy has also been used to determine moisture content in porcine meat (Yang et al., 2013). This meat, which has a high moisture content, allows microbes to multiply easily, which may cause meat spoilage. Several porcine pieces were evaluated by a four-terminal electrode portable impedance spectroscopy system. The results indicated a good relationship between impedance parameters and moisture content, as determined by standard chemical methods.

### 20.3.3.2 Fish Spoilage Studies

Impedance spectroscopy was one of the first electrochemical techniques applied to determine fish spoilage; indeed, an electronic device able to measure changes in impedance at different frequencies was used for measuring fish freshness some decades ago (Jason and Richards, 1975). Niu and Lee (2000) used impedance spectroscopy in fish samples and correlated data from dielectric properties with physicochemical parameters. These authors used a frequency sweep ranging from 0.1 to 100 kHz to measure dielectric changes in various fish species (ie, carp, herring, and sea bass) while the fish were stored after death. The authors


Sample
v(t)
Equivalent impedance
Z
i(t)
— v(t)
— i(t)
|v(t)|
|i(t)|
Δt
t
Frequency $f_1$
$Z_1 = |Z_1| e^{j\varphi_1}$
Complex number
$|Z_1| = M_1 = \frac{|v(t)|}{|i(t)|}$
Module for $f_1$
$\varphi_1 = 2\pi f_1 \Delta t$
Phase for $f_1$
Frequency $f_2$
$Z_2 = |Z_2| e^{j\varphi_2}$
Complex number
$|Z_2| = M_2 = \frac{|v(t)|}{|i(t)|}$
Module for $f_2$
$\varphi_2 = 2\pi f_2 \Delta t$
Phase for $f_2$
Frequency $f_n$
$Z_n = |Z_n| e^{j\varphi_n}$
Complex number
$|Z_n| = M_n = \frac{|v(t)|}{|i(t)|}$
Module for $f_n$
$\varphi_n = 2\pi f_n \Delta t$
Phase for $f_n$
Impedance plots
|Z|
Module plot
$M_1$ $M_2$
$M_n$
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φ
Phase plot
$\varphi_1$ $\varphi_2$
$\varphi_n$
$f_1$ $f_2$ . . . $f_n$ $f$

**FIGURE 20.4** **Impedance spectroscopy measurement process.**

found that changes in the phase angle and admittance were the best freshness indicators. Based on these studies, four freshness classifications were defined for all the tested fish species. Using virtual instrument technology and an impedance analysis, Zhang et al. (2009) developed a measurement system to study freshwater fish silver carp during storage (180 h) with an ANN model.

One proposed way to summarize impedance studies is to use the *Q*-value, $Q = (Z_L - Z_H)100/Z_H$, where $Z_L$ and $Z_H$ are impedances of samples at certain frequencies (usually 1 and 16 kHz, respectively) measured at two different voltages (6 and 9 V). A high correlation between the *Q*-value and certain parameters was assessed and used to determine fish freshness (Zhang et al., 2011). They also employed the *Q*-value to differentiate fresh fish from frozen-thawed fish. Studies on two freshwater fish species lasting 10 days were carried out. This author found that the impedances of fresh and frozen-thawed fish decreased as frequency increased,

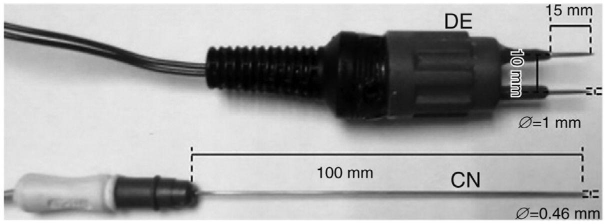
DE
15 mm
10 mm
Ø=1 mm
100 mm
CN
Ø=0.46 mm

**FIGURE 20.5** **Impedance spectroscopy electrodes: double electrode (DE) and coaxial needle electrode (CN).**

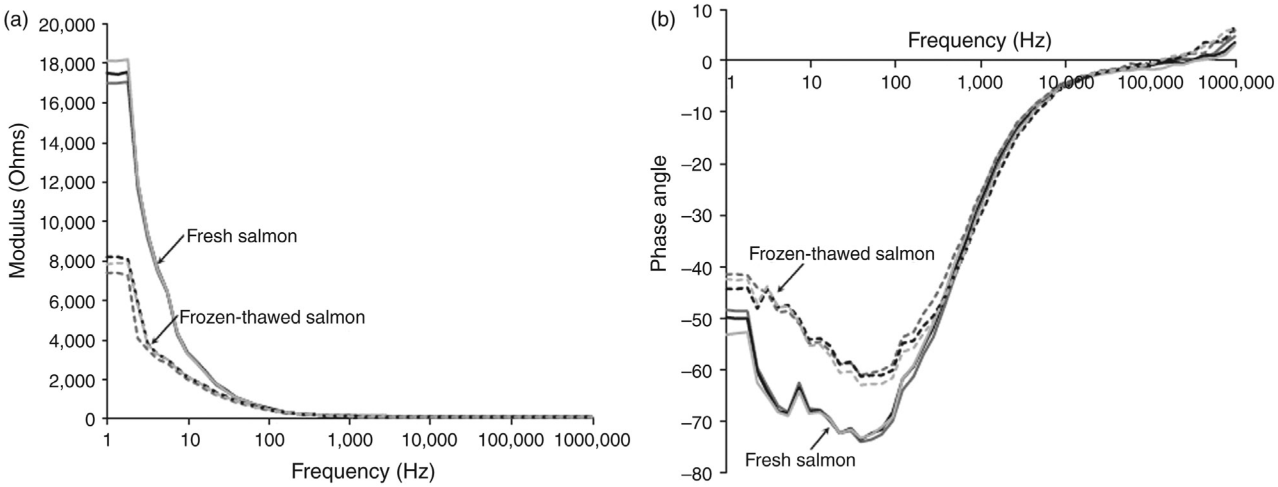

**FIGURE 20.6** (a) Module values and (b) phase values of the impedance spectrum of fresh salmon and frozen-thawed salmon samples.

and that *Q*-values lowered as storage time increased. However, the *Q*-values of fresh fish were higher than those of frozen-thawed fish (Zhang et al., 2010). This method was also used to achieve a correlation between the electrical conductivity of a gutted fish body and bighead carp quality (*Aristichthys nobilis*) stored at 0 and 3°C (Zhu et al., 2013).

Using the impedance spectroscopy equipment developed by some of us (*vide ante*), several assays have been performed to differentiate between fresh and frozen-thawed fish. Samples of fresh salmon and others frozen at −18°C or for two freezing cycles were analyzed (Fernández-Segovia et al., 2012). In general, no significant differences in moisture, total volatile basic nitrogen, pH, texture parameters, *K*-value, or microbial counts between the different samples were observed. This revealed that the freezing process, storage time, or number of freezing cycles did not affect the physicochemical parameters of fish samples, except for water-holding capacity, which was significantly lower in all the frozen samples versus fresh salmon. The authors found that it was possible to differentiate fresh salmon from frozen-thawed samples by taking impedance measurements. However, no discrimination was achieved for the samples stored at −18°C or for those submitted to different freezing cycles. They also suggested that impedance spectroscopy can be used to assess damage to fish tissue as a result of the freezing process, and to permit the detection of fraud, for example, frozen-thawed products sold as fresh fish.

A similar goal, that is, differentiating between fresh and thawed fish, has been pursued using two different electrodes (ie, a double electrode and an arrowhead electrode) (Fuentes et al., 2013). Fuentes and coworkers found that the freezing process did not affect moisture, pH, TVB-N, or microbial quality. However, it provoked a slight degradation of IMP, a slight increase in the TBA index, and reduced water-holding capacity; it also affected several textural parameters. All these changes were also detected with impedance spectroscopy measurements using the double electrode. In fact, the system was able to differentiate fresh sea bream from frozen-thawed samples (Fig. 20.6). In contrast, arrowhead electrodes were unable to discriminate between different sample types. The frozen storage time had no effect on either the evaluated microbial and physicochemical parameters or the electrical properties of muscle. The potential use of electrical impedance has also been investigated to differentiate thawed sea bass fillets (*Dicentrarchus labrax*) that had been previously subjected to different freezing conditions (Vidaček et al., 2012).

Impedance spectroscopy has been recently used for predicting freshness of sea bream (Pérez-Esteve et al., 2014). The system was particularly able to classify raw matter into six groups according to composition differences, and to classify those samples stored for times of between 0 and 15 days into different groups according to degree of freshness. Different physical and chemical parameters (ie, moisture, fat, pH, and TVB-N) were also determined. The PLS statistical analyses allowed the creation of a model that correlated impedance data with TVB-N content.

The previous examples were run at low frequencies (up to 1 MHz). However, attempts to use higher frequencies have also been made. In particular, it has been reported that deteriorative biochemical and microbiological processes during the gradual spoilage of meat and fish tissue can influence the dielectric properties in a microwave frequency region. In this region, the complex interactions of water, solutes, and structure-forming proteins are systematically changed by death and decay (Kent et al., 2007). By way of example, Castro-Giráldez et al. (2010) used the dielectric spectra within the microwave range (0.5–20 GHz) to detect quality defects in postmortem porcine muscle. This study revealed that dielectric properties at two frequencies

(0.5 and 10 GHz) proved to be a useful tool for determining meat quality classes soon after slaughter.

## 20.4 CONCLUSIONS

This chapter provides a description of works on the application of e-tongues to monitor freshness and spoilage of meat and fish samples. Despite the undoubted interest shown in the food industry, these applications have not become commonplace given the difficulty of taking measurements on solid foods. Nevertheless, research works have demonstrated that the use of electronic tongues in this field is potentially strong. Of the three electrochemical techniques discussed in this chapter, impedance spectroscopy offers more examples and has obtained the best results, especially for fish freshness monitoring. In most cases, a good correlation between the physicochemical and microbial parameters and the data obtained from e-tongues has been obtained, which strongly suggests that this technique can be a useful tool in assessing the shelf life of meat and fish samples.

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## Chapter 21

# Milk and Dairy Products Analysis by Means of an Electronic Tongue

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## 21.1 INTRODUCTION

Electronic tongues (e-tongues)[a] are sensor arrays coupled with numerical procedures, which are capable of distinguishing various liquids employing the concept of partial selectivity/cross-sensitivity. The pattern of the sensor array responses serves as a fingerprint or barcode for the analyzed sample. The identification of the measured samples is usually a classification task; therefore, there has been a trend to use artificial intelligence and information visualization methods to extract important information from sensor responses (Ciosek and Wróblewski, 2007a; Riul et al., 2010). Most e-tongues, both commercial versions as well as those in the R&D phase (laboratory versions), are composed of three elements: an automatic sampler (although it is not a necessary component), an array of chemical sensors with different selectivity patterns, and software in a form of an appropriate algorithm for processing the obtained signals and revealing the results of analysis (Fig. 21.1; Peris and Escuder-Gilabert, 2013).

Such architecture of these systems allows for many successful applications in the analysis of various foodstuffs such as wines, fruit juices, coffee, milk, and beverages (Escuder-Gilabert and Peris, 2010). Among those, especially challenging are samples with high protein content, like milk and milk products, which can affect sensor performances. Nevertheless, until now, there have been numerous attempts to analyze milk and dairy products by means of e-tongue systems (Table 21.1), as they are a very attractive alternative to traditionally used techniques because they are related with the low cost of analysis, the simplicity of measurements, and applicability to online conditions. Such applications include the analysis of taste and flavor, freshness evaluation, microbial growth monitoring, origin recognition, adulteration detection, quality control studies, and process monitoring—they are all reviewed and discussed in the following sections of this chapter.

## 21.2 ANALYSIS OF TASTE AND FLAVOR

The first publication on electronic tongue dates back to 1990, when Toko and coworkers described so-called multichannel taste sensor (Hayashi et al., 1990; Fig. 21.1). Five years later the same group also presented for the first time application of e-tongue technology to milk analysis (Toko et al., 1995). Taste sensor with global selectivity was composed of several kinds of lipid/polymer membranes for transforming information about taste substances into an electric signal. The output showed various pattern responses for chemical substances of different taste qualities, such as saltiness, bitterness, and sourness (Toko, 1998; Fig. 21.2).

The taste of milk could be therefore characterized quantitatively, providing the objective scale for the human sensory expression. The output of the taste sensor showed high correlations with richness determined by the human panel and the degree of protein denaturation (Toko et al., 1995). In the following publications, the same device was used for taste analysis of milk samples prepared under various conditions of homogenization or UHT treatment. Milk heated at 100°C for different processing times (0, 1, 5, 15, 30 min) was successfully discriminated using the taste senor, whereas significant discrimination was possible between only two, 0 and 30 min, treatments by the human sensory evaluations (Toko, 1996). Another example of higher sensitivity of a taste sensor in comparison to the human sense of taste was the detection of slight changes occurring in milk during homogenization (Yamada et al., 1997). Sensory evaluation by the human panel for expressing "richness" showed that there were no significant differences among the milk samples treated with different homogenization pressure. On the other hand, it was possible to discriminate the samples by means of the taste sensor because its response patterns were very different below and above 100 $kg/cm^2$ of the homogenization pressure, which was related with the different distribution of the fat globule size below and above

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[a] All abbreviations are explained in Table 1.

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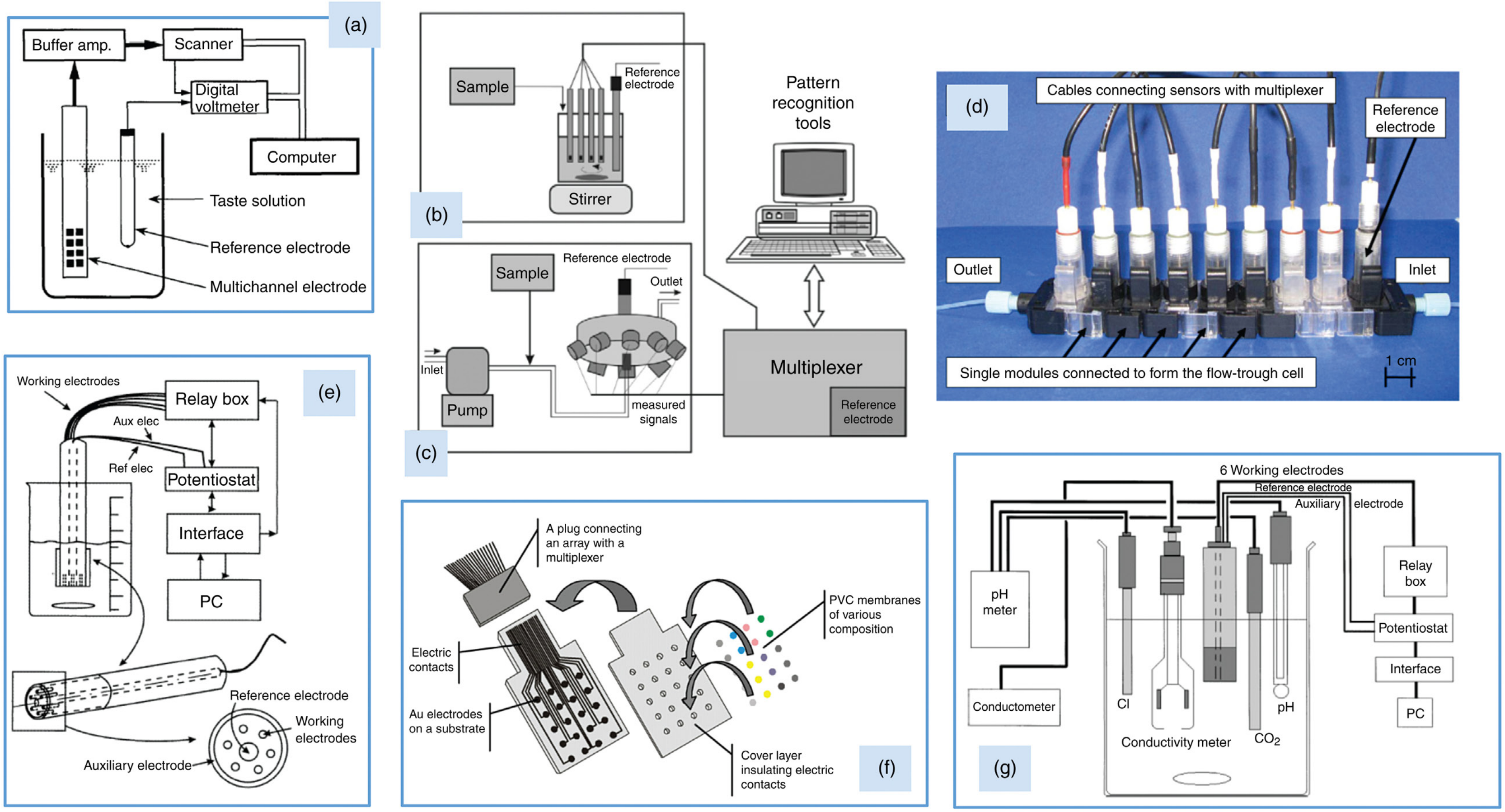
(a)
Buffer amp.
Scanner
Digital voltmeter
Computer
Taste solution
Reference electrode
Multichannel electrode
(b)
Sample
Reference electrode
Stirrer
(c)
Sample
Reference electrode
Outlet
Inlet
Pump
measured signals
Pattern recognition tools
Multiplexer
Reference electrode
(d)
Cables connecting sensors with multiplexer
Reference electrode
Outlet
Inlet
Single modules connected to form the flow-trough cell
1 cm
(e)
Working electrodes
Relay box
Aux elec
Ref elec
Potentiostat
Interface
PC
Reference electrode
Working electrodes
Auxiliary electrode
(f)
A plug connecting an array with a multiplexer
PVC membranes of various composition
Electric contacts
Au electrodes on a substrate
Cover layer insulating electric contacts
(g)
6 Working electrodes
Reference electrode
Auxiliary
electrode
pH meter
Relay box
Potentiostat
Interface
PC
Conductometer
Cl
pH
Conductivity meter
CO2

**FIGURE 21.1 Various e-tongue setups used for milks and dairy products analysis.** (a) Taste sensor based on multichannel electrode; (b) ISE array for batch measurements; (c) Flow-through cell for SSE array; (d) Modular flow-through cell with miniaturized ISEs; (e) Voltammetric cell based on various metal working electrodes; (f) Integrated array of SSEs; (g) Hybrid e-tongue. *(Adapted from Toko, 1996; Winquist et al., 1998,© IOP Publishing. Reproduced with permission. All rights reserved; Winquist et al., 2000; Ciosek and Wróblewski, 2007b, 2008; Witkowska et al., 2010.)*

**TABLE 21.1** Analysis of Various Milk Samples and Dairy Products by Means of E-Tongues

| Sensing Unit | Pattern Recognition Procedure | Application | References |
|---|---|---|---|
| **Potentiometric e-tongues** | | | |
| Taste sensor—set of electrodes with lipid membranes | Direct analysis of potentiometric response | Quantitative description of taste of milk as an effect of heat treatment for pasteurization/sterilization purposes | Toko et al. (1995) |
| Taste sensor—multichannel electrode with lipid membranes | Direct analysis of potentiometric response, PCA | Quantitative description of taste of milk | Toko (1996, 1998, 2000, 2004) |
| Taste sensing system SA401 (Anritsu Co., Lt, Japan) | Direct analysis of potentiometric response, PCA | Discrimination of milk samples treated with different homogenization pressure | Yamada et al. (1997) |
| Taste sensor—multichannel electrode with lipid membranes | Direct analysis of potentiometric response | Taste of UHT milk after various exposure to light | Mizota et al. (2009) |
| Taste sensor—multichannel electrode with lipid membranes | Direct analysis of potentiometric response | Taste of milk from breastfeeding mothers with or without mastitis | Yoshida et al. (2014) |
| Taste sensing system SA402B (Intelligent Sensor Technology, Japan) | Direct analysis of potentiometric response | Taste of bovine milk whey protein | Sano et al. (2005) |
| Screen-printed multichannel taste sensor | PCA | Discrimination between fresh and spoiled milk, monitoring of milk quality deterioration during storage | Sim et al. (2003) |
| ISE array (ion-selective and partially selective electrodes) | Direct analysis of potentiometric response patterns, PCA, ANN | Classification of milks according to brand/dairy origin | Ciosek et al. (2004) |
| SSE array in flow-through cell (ion-selective and partially selective electrodes) | Direct analysis of potentiometric response patterns, PCA, ANN, PLS-DA | Classification of milks according to brand/dairy origin | Ciosek and Wróblewski (2007b); Ciosek et al. (2006b) |
| SSE array in flow-through cell (ion-selective and partially selective electrodes) | Direct analysis of potentiometric response patterns, PCA, SVM network | Classification of milks according to brand/dairy origin, discrimination of milks of various fat content | Ciosek et al. (2006a) |
| ISE array (ion-selective and partially selective electrodes) | Direct analysis of potentiometric response patterns, PCA, *k*-NN, PLS, SIMCA, BPNN, PNN, LVQ network | Classification of milks according to brand/dairy origin | Ciosek and Wróblewski (2006); Ciosek et al. (2006c) |
| Integrated array of SSEs | Direct analysis of potentiometric response patterns, PLS-DA | Classification of milks according to brand/dairy origin | Ciosek and Wróblewski (2008) |
| SSE array in modular flow-through cell (ion-selective and partially selective electrodes) | PLS | Monitoring of methane fermentation with whey as a substrate: discrimination between fermentation times, prediction of chemical oxygen demand and volatile fatty acid contents | Buczkowska et al. (2010) |
| ISE array in modular flow-through cell (ion-selective and partially selective) | PLS | Monitoring of methane fermentation with whey as a substrate: discrimination between fermentation times, prediction of chemical oxygen demand and volatile fatty acid contents | Witkowska et al. (2010) |
| ISE array | PLS | Monitoring of fermentation process of starting culture for light cheese production: detection of "abnormal" operating conditions, determination of citric, lactic, and orotic acid in the fermentation media, determination of peptide profiles | Esbensen et al. (2004) |

(*Continued*)

**TABLE 21.1** Analysis of Various Milk Samples and Dairy Products by Means of E-Tongues (*cont.*)

| Sensing Unit | Pattern Recognition Procedure | Application | References |
|---|---|---|---|
| ISE array | PCA, SIMCA, LDA | Discrimination between milks from healthy and infected with bovine mastitis glands | Mottram et al. (2007) |
| ISFET array (α-Astree e-tongue, Alpha MOS, France) | PCA, CDA | Soya milk discrimination | Kovács et al. (2009) |
| ISFET array (α-Astree e-tongue, Alpha MOS, France) | PCA | Recognition of different milk and yogurt samples from different producers, discrimination between various dairy products from one manufacturer | Hruškar et al. (2009) |
| ISFET array (α-Astree e-tongue, Alpha MOS, France) | PCA, ANN, PLS | Monitoring of changes in probiotic fermented milk during storage, classification of probiotic fermented milk according to flavor and taste | Hruškar et al. (2010b) |
| ISFET array (α-Astree e-tongue, Alpha MOS, France) | ANN | Determination of ethanol, acetaldehyde, diacetyl, lactic acid, acetic acid, and citric acid content in probiotic fermented milk | Hruškar et al. (2010a) |
| ISFET array (α-Astree e-tongue, Alpha MOS, France) | DFA | Discrimination between coconut milk protein powder samples stored at different conditions | Naik et al. (2013) |
| ISFET array (α-Astree e-tongue, Alpha MOS, France) | PLS, PCA | Estimation of bitter taste of dairy protein hydrolysates | Newman et al. (2014a,b) |
| SCE array | PCA, LDA | Detection of goat milk adulteration with bovine milk | Dias et al. (2009) |
| **Voltammetric e-tongues** | | | |
| Voltammetric cell with 2 working electrodes (Au, Pt), application of large and small amplitude pulsed voltammetry | PCA | Discrimination between milks and other beverages, monitoring of aging processes of milk when stored at room temperature | Winquist et al. (1997) |
| Voltammetric cell with 5 working electrodes (Au, Ir, Pd, Pt, Rh) application of pulsed voltammetry | PCA, PLS, ANN | Monitoring of deterioration of the quality of milk due to microbial growth when stored at room temperature | Winquist et al. (1998) |
| Voltammetric e-tongue | Wavelet transform, PCA, MDC | Monitoring of bacteriological growth | Robertsson and Wide (2004) |
| Voltammetric cell with 4 working electrodes (Au, Pt, Rh, stainless steel) | PCA | Process control in dairy industry: detection of different sources of milk coming into the process, monitoring of cleaning process | Winquist et al. (2005) |
| Voltammetric cell with 2 working electrodes (Au and Au modified with a Prussian blue film), application of cyclic voltammetry | PCA | Evaluation of milk adulteration, discrimination between milks of various fat content | Paixão and Bertotti (2009) |
| Voltammetric cell with 5 working electrodes (Au, Pt, Ag, Pd, Ti), application of MLAPV | PCA, DFA, PCR, PLS, LS-SVM | Detection of antibiotic residues in bovine milk, determination of concentration of antibiotics | Wei and Wang (2011) |
| Voltammetric cell with 4 working electrodes (Au, Ag, Pt, Pd), application of MRPV and MSPV | PCA, CA, PLS, LS-SVM | Monitoring of quality change and storage time of unsealed pasteurized milk, estimation of bacterial count, acidity and viscosity changes during storage | Wei et al. (2013b) |

| | | | |
|---|---|---|---|
| Voltammetric cell with 6 working electrodes (Au, Pt, Pd, W, Ti, Ag), application of MLAPV | PCA, Kernel PCA, LLE, Sammon mapping | Classification of milk powder solutions by means of the storage time | Tian et al. (2013) |
| Voltammetric cell with 4 working electrodes (Au, Ag, Pt, Pd), application of MHPV, MRPV, MSPV | PCA, DFA, PLS-DA | Discrimination between various categories of set yogurt | Wei et al. (2013a) |
| **Other sensing principles: e-tongues based on acoustic sensors and e-tonges with SPR detection** | | | |
| SH-SAW array | PCA | Discrimination between milk samples with different fat content, monitoring of aging processes of milk | Cole et al. (2002) |
| SH-SAW array | PCA | Analysis the bacterial load in cow's milk | Gardner (2005) |
| Array of combinatorial cross-reactive receptors with SPR detection | PCA | Discrimination among animal-based and plant-based milks, deterioration of UHT milk quality | Genua et al. (2014); Garçon et al. (2014) |
| **Sensor fusion: hybrid e-tongues and combined e-tongue and e-nose systems** | | | |
| Hybrid e-tongue—combination of conductivity, potentiometry (pH, $CO_2$, $Cl^-$ ISEs) and voltammetry (6 working electrodes: Au, Ir, Pd, Pt, Re, Rh) | PCA, ANN | Classification of milks fermented by various types of microorganisms | Winquist et al. (2000) |
| Combined e-tongue (SH-SAW) and E-nose (ChemFETs) system | PCA | Discrimination of milks according to fat level | Cole et al. (2011) |
| Combined E-tongue (voltammetric cell with 4 working electrodes: Pt, Au, glassy carbon, Ag) and E-nose (8 MOS sensors) system | PCA, SVM | Determination of aging time and brand of milks | Bougrini et al. (2014) |
| Combined E-tongue (Taste Sensing System SA402, Intelligent Sensor Technology, Japan) and E-nose (Fox 3000, Alpha MOS, France) system | Direct analysis of potentiometric response , PCA | Sensory attributes of raw milk and UHT milk | Mizota et al. (2008) |

Abbreviations: SSE, solid-state electrode; SCE, solid-contact electrode; SH-SAW, shear horizontal surface acoustic wave sensor, MLAPV, multifrequency large-amplitude pulse voltammetry; MRPV, multifrequency rectangle pulse voltammetry; MSPV, multifrequency staircase pulse voltammetry; MHPV, multifrequency hackle pulse voltammetry; PCA, principal components analysis; PCR, principal components regression; ANN, artificial neural network; SVM, support vector machine; *k*-NN, *k*-nearest neighbor; PLS, partial least squares; PLS-DA, PLS-discriminant analysis; SIMCA, soft independent modeling of class analogy; BPNN, back-propagation neural network; LVQ, learning vector quantization; PNN, probabilistic neural network; LDA, linear discriminant analysis; CDA, canonical discriminant analysis; DFA, discriminant function analysis; MDC, minimum distance classifier; LS-SVM, least squares-SVM; LLE, locally linear embedding.

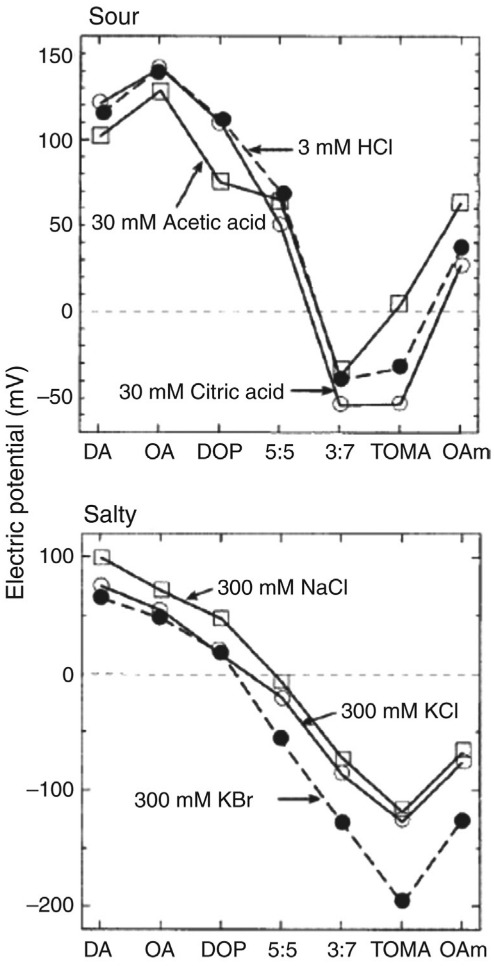

**FIGURE 21.2 Taste sensor response patterns toward sour and salty substances.** *(Adapted from Toko, 1998 © IOP Publishing. Reproduced with permission. All rights reserved.)*

this pressure. Similar sensitivity toward taste changes was observed in the case of temperature treatment. Five types of milk, including ultra-high temperature (UHT) processed milk and four types of reconstituted milk were prepared by combining raw milk, skim milk powder, and butter. Different pasteurization methods (indirect heating and direct heating) were performed, which resulted in types of milk that were analyzed by a taste sensor, an odor sensor, and sensory evaluation. It was observed that, to add richness, it was effective to use raw milk and use a plate-type UHT pasteurizer with indirect heating. To give a finish with a plain flavor, it was effective to use a steam infusion-type UHT pasteurizer with direct heating. Correlation between the "cooked flavor" and "saltiness" determined by the human panel and the taste sensor output was noticed (Mizota et al., 2008). Influence of light exposure, a physical factor, on the taste of milk was investigated in Mizota et al. (2009). UHT processed milk packaged in cartons of five different colors had been stored under fluorescent light for 10 days, and then evaluated by the human panel and instrumental analysis. The results of the sensory evaluation confirmed that black carton was the most effective in preventing off-flavor due to light and that the milk in the white carton had the highest off-flavor. No difference in the flavor was found in the case of milk stored in red, blue, and green cartons. The correlation between taste sensor response values and sensory evaluation scores or hexanal amounts determined by GC–MS was high. It was suggested that hexanal content is an effective indicator of off-flavor, which can be objectively evaluated by taste sensor analysis (Mizota et al., 2009).

Taste assessment with the use of e-tongue technology was performed also for other kinds of milk than bovine milk; human milk and soya milk (Yoshida et al., 2014; Kovács et al., 2009). The refusal of infants to suckle from a breast that is inflamed with mastitis suggests that the taste of the milk is changed. The taste of milk from breast-feeding mothers with or without mastitis was compared in Yoshida et al. (2014). The intensity of four basic tastes—sourness, saltiness, bitterness, and umami—of breast milk from 24 healthy mothers and from 14 mothers with mastitis was determined objectively using a taste sensor. It was found that the transition from colostrum to mature milk was accompanied by changes in the taste of the milk, such as decreased saltiness and umami and increased bitterness and sourness. Umami and saltiness increased in milk from inflamed breasts because contents of sodium, glutamate, and guanosine monophosphate were increased in such cases (Yoshida et al., 2014).

The objective of the research described in Kovács et al. (2009) was to compare the taste attributes of different commercial soya drinks and to determine the effect of different ingredients and processing technologies on the taste attributes of soya products. The results of the α-Astree e-tongue (Alpha MOS, France) measurements revealed that this instrument is able to determine the effect of the applied technology and to distinguish soya milk samples according to sensory preferences. It also showed that the taste attributes of soya juice made from hulled soybeans was beneficial for the taste attributes relative to that of the juice made of not-hulled soybeans.

Probiotics, which are living nonpathogenic microorganisms exerting a positive influence on the host's health or physiology, can be obtained in a form of probiotic fermented milk. Taste of such products plays a crucial role in consumer preferences. According to that, research for the objective characterization of milk probiotics taste was conducted (Hruškar et al., 2010a,b). For classification of probiotic fermented milk according to flavor, the α-Astree e-tongue was applied. The samples of plain, strawberry, apple-pear, and forest-fruit probiotic fermented milk were stored for 20 days at 2 different temperatures and monitored by the e-tongue and a human sensory panel. Various pattern recognition techniques were adapted for the analysis of sensor array responses: principal components analysis (PCA) for monitoring changes occurring in probiotic fermented milk in time; artificial neural network (ANN) for the classification of probiotic fermented milk during storage and according to flavor; and partial least squares (PLS) and ANN to estimate and predict the sensory panel evaluation results and thus the quality of the probiotic fermented milk measured by consumer (Hruškar et al., 2010b). The same research group reported in (Hruškar et al., 2010a) the application of the same instrument for simultaneous determination of ethanol, acetaldehyde, diacetyl, lactic acid, acetic acid, and citric acid content in probiotic fermented milk—the same type samples as in (Hruškar et al., 2010b). The highest correlation (0.967) and lowest standard deviation of error for the training and testing subsets were obtained for the estimation of ethanol content. The ANN models for acetic acid, citric acid, lactic acid, and diacetyl concentration determination also exhibited good prediction capability with slightly higher prediction errors. The model for acetaldehyde determination exhibited low accuracy of prediction, which was most likely caused by low sensitivity of the potentiometric sensor array to acetaldehyde. Therefore, it was concluded that this method exhibited great potential as a tool in the rapid determination of aroma compounds in probiotic fermented milk (Hruškar et al., 2010a).

Bovine milk whey protein is produced as a by-product from cheese and butter manufacturing and can be used for food processing and as an additive for nutritional fortification (it has high nutritional value; Sano et al., 2005). It elicits no taste stimulation; however, it interacts with various flavor compounds. This effect was investigated with the use of the taste sensing system SA402B (Intelligent Sensor Technology, Japan). It was confirmed that the astringency of these proteins increased with the increase in protein concentration, and they elicited strong astringency at 10 mg/mL under acidic conditions. The taste sensor gave specific values for whey proteins at pH 3.5, which corresponded well to those obtained by the sensory analysis. Elicitation of astringency induced by whey protein under acidic conditions is probably caused by aggregation and precipitation of protein molecules in the mouth (Sano et al., 2005).

Bovine milk proteins obtained in dairy industry can be processed in order to improve their properties (eg, they can be hydrolyzed). The incorporation of dairy protein hydrolysates (DPH) into foods has numerous benefits over nonhydrolyzed protein because they exhibit improved solubility and gelatin-forming abilities, and are a rich source of bioactive peptides. However, such additions can elicit bitterness that can develop as a result of the hydrolysis process (due to the alteration of the native protein structures to yield short-chained peptides with exposed hydrophobic amino acids). This phenomenon can be evaluated by sensory analysis using a human taste panel; however, it can present difficulties in implementation during the research and development phase of DPH products. The difficulties are that the process is time consuming and needs a large quantity of food grade to sample, which is difficult in the early stage of laboratory development. There is also a risk of microbial or chemical contamination at the lab production level. As an alternative, an e-tongue could be employed. First, the potential of the α-Astree e-tongue to be used in bitterness screening of various DPHs was shown by Newman et al. (2014a). Later, 19 DPHs were analyzed by e-tongue, size exclusion chromatography, and reverse phase HPLC and the results were correlated with their bitterness intensity as scored by a trained sensory panel. It was found that PLS models constructed with the e-tongue and HPLC data had the potential to be used for prediction of bitterness and thus reducing the reliance on sensory analysis in DPHs for future food research (Newman et al., 2014b).

## 21.3 FRESHNESS EVALUATION, AGING, AND MICROBIAL GROWTH MONITORING

A few years after the appearance of potentiometric sensor arrays used mainly for taste evaluation, the voltammetric e-tongue was presented by Winquist et al. (1997, 1998). They pointed out that voltammetry had been extensively used in analytical chemistry due to several important advantages: very high sensitivity, versatility, simplicity, and robustness. Besides, this technique offers a wide range of various measurement modes, including cyclic, stripping, and pulse voltammetry, as well as the possibility of using different types of metals for the working electrode. Depending on the techniques and electrodes used, various aspects of information on the measured sample can be obtained (Winquist et al., 1997). The voltammetric e-tongue based on five wires of different metals as working electrodes, reference and auxiliary electrodes, to which pulsed voltammetry was applied, were allowed to follow the deterioration of the quality of milk due to microbial growth when milk was stored at room temperature. The deterioration process could clearly be followed on the PCA plot (Fig. 21.3). However, it was emphasized that there are many phenomena occurring during the storage of milk apart from microbial growth, which could affect the performandt: the oxidation of fatty acids, the evaporation of volatile compounds, and the adsorption of proteins to the electrode surface. Nevertheless, the prediction of the course of bacterial growth in the milk samples with the use of PLS and ANNs provided satisfactory correctness (Winquist et al., 1998).

The problem of extracting the important information from a complex response of a voltammetric e-tongue was addressed in Robertsson and Wide (2004). For this purpose, a wavelet transform was used—the amount of data to be analyzed was significantly reduced without loss of important information. The obtained approximation coefficients, extracted as features, were used for classification with the use of a minimum distance classifier (MDC), which led to appropriate monitoring of bacteriological growth in milk (Robertsson and Wide, 2004). The classification ability of the voltammetric e-tongue can be also enhanced with the use of appropriate measurement mode [eg, multifrequency rectangle pulse voltammetry—MRPV, multifrequency staircase pulse voltammetry—MSPV (Wei et al., 2013b); multifrequency large-amplitude pulse voltammetry—MLAPV (Tian et al., 2013)]; and appropriate classifiers

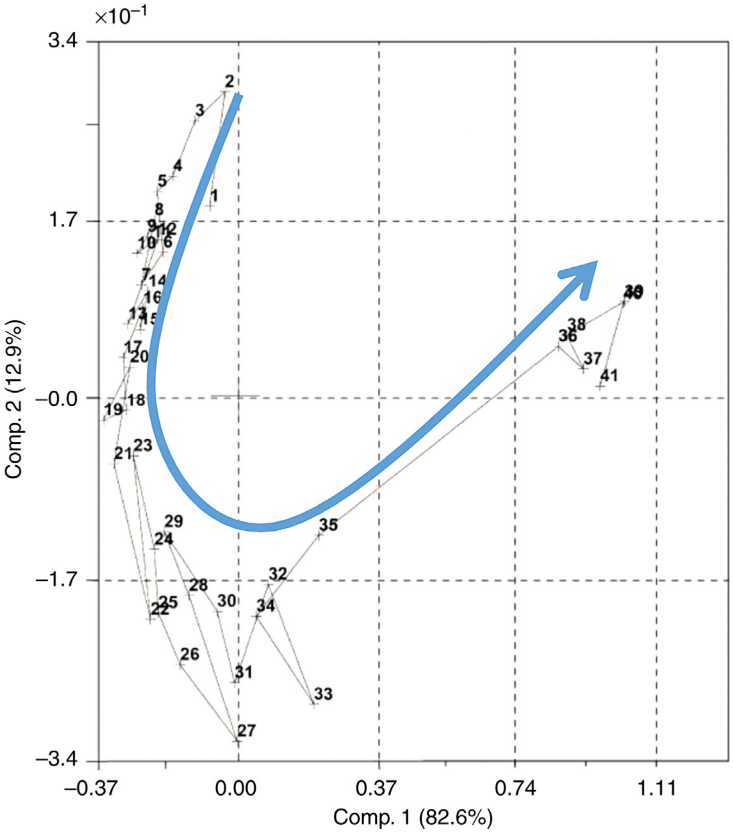

**FIGURE 21.3 Monitoring of aging process of milk by voltammetric e-tongue.** *(Adapted from Winquist et al., 1997.)*

[eg, least squares-support vector machine—LS-SVM (Wei et al., 2013b), Kernel PCA, locally linear embedding—LLE, Sammon mapping (Tian et al., 2013)]. Voltammetric cell with four working electrodes was applied to monitor the quality and storage time of unsealed pasteurized milk (Wei et al., 2013b). Two potential waveforms, MRPV and MSPV, were applied to the working electrodes in this study. Total areas under the corresponding curves were applied as characteristic data, which were evaluated by PCA and CA. The results indicated that the milk samples of different storage time could be successfully classified. Furthermore, the total bacterial count and viscosity properties were predicted by PLS analysis and LS-SVM and better results were obtained by the latter one (Wei et al., 2013b). The voltammetric e-tongue based on MLAPV was also applied to the classification of five milk powder solutions by means of the storage time. To the analysis of the obtained data, three nonlinear multivariate data analysis methods were proposed: Kernel PCA, LLE, and Sammon mapping (Tian et al., 2013). The results indicated that the three nonlinear procedures were able to extract the useful information from the raw data and thus exhibited a better performance than PCA, so they can be promising for voltammetric e-tongue data processing (Tian et al., 2013).

For monitoring of the aging processes of various milks, potentiometric sensor arrays were also applied; the α-Astree e-tongue (Naik et al., 2013; Hruškar et al., 2010b) and the screen printed disposable taste sensor (Sim et al., 2003). The latter was used to analyze two types of commercial milk; UHT and pasteurized milk. This device was found to be able to discriminate reliably between fresh and spoiled milk and to follow the deterioration of the milk quality when it was stored at room temperature (Sim et al., 2003). The storage study of coconut protein powder (CPP) was performed with an α-Astree e-tongue (Naik et al., 2013). Coconut skim milk and insoluble protein are two major by-products in the production of virgin coconut oil. Coconut skim milk was homogenized along with insoluble protein and spray dried to obtain a value-added product—CPP. The samples were kept under different conditions (refrigerated [control], ambient and accelerated), and withdrawn periodically at designated intervals of 15 or 30 days. The e-tongue showed no significant difference in attributes of CPP during the storage period of 2 months (Naik et al., 2013). The same e-tongue was applied to the monitoring of changes in probiotic fermented milk during storage (Hruškar et al., 2010b, described in Section 21.2).

The design and characterization of shear horizontal surface acoustic wave (SH-SAW) devices for the analysis of liquid samples were described in Cole et al. (2002) and Gardner (2005). They were fabricated on $LiTaO_3$ and $LiNbO_3$ substrates. The design consisted of a dual delay line configuration where one delay line is metallized and shielded and the other is left electrically active. Simultaneous measurements of both mechanical properties and electrical parameters of the liquid under test are therefore achieved. Apart from mass loading and viscosity, it is possible to determine permittivity and conductivity of the liquid under test. These parameters can be related to taste properties. The analysis of signals of SH-SAW devices revealed discrimination between fresh and spoiled milk on a PCA plot (Cole et al., 2002) and allowed for determination of bacterial load in cow's milk (Gardner, 2005).

Recently, a new type of e-tongue based on SPR was presented (Genua et al., 2014; Garçon et al., 2014). It employs an array of nonspecific and cross-reactive combinatorial receptors prepared by mixing two small molecules in varying and controlled proportions and allowing the mixtures to self-assemble on the SPR prism surface (Genua et al., 2014). Using only two small molecules as building blocks (lactose and sulfated lactose), an array of combinatorial cross-reactive receptors was prepared and combined with an optical detection system—SPR imaging. The obtained device generated unique 2D continuous evolution profiles and 3-D continuous evolution landscapes, based on which device the differentiation of complex mixtures was performed with the use of PCA. The preliminary experiments that were devoted to the monitoring of the deterioration of UHT milk in time demonstrated its potential for quality control applications (Genua et al., 2014).

A combined approach based on a two multisensor systems (the hybrid e-nose and a voltammetric e-tongue) was presented in Bougrini et al. (2014). Additional chemical information on the sample is gained in this case thanks to the analysis of both the solution and its headspace. The aim of this system was exact recognition of storage time of milk with the use of the support vector machine (SVM). Results obtained by the data fusion approach outperformed the classification results of the e-nose and the e-tongue taken individually. This study can be generalized to various food products where quality is based on the perception of both taste and flavor (Bougrini et al., 2014).

## 21.4 FERMENTATION MONITORING AND ANALYSIS OF FERMENTED MILK PRODUCTS

The fermentation of dairy foods represents one of the oldest techniques for food preservation. Fermented milk products, that is, cultured milk products, are dairy foods fermented with various lactic acid bacteria. This process not only increases the shelf-life of the product, but also allows for enhancing the taste and improves the digestibility of milk. However, fermentation processes are often sensitive to even slight changes of conditions that may result in unacceptable end-product quality. Therefore, detecting unfavorable deviations from a normal process run should be fulfilled as early as possible because of economic reasons. For this purpose, various traditional analytical techniques can be employed, which, however, need expensive instrumentation, experienced operators, and complex sample preparation. As an alternative multisensor system, including e-tongues can be used for allowing rapid and relatively inexpensive quality control during fermentation process (Peris and Escuder-Gilabert, 2013).

One example is the e-tongue based on potentiometric sensor array that was applied to qualitative and quantitative monitoring of a batch fermentation process of starting culture for light cheese production (Esbensen et al., 2004). Process control charts were built on the basis of sensor array output combined with PLS, while measuring sensor responses during a standard fermentation run. Control charts were allowed then to detect samples from fermentation batches running under "abnormal" operating conditions (created by setting process parameters outside recommended limits). Moreover, the high capability of the e-tongue to quantify concentrations of important organic acids (citric, lactic, and orotic) in the investigated fermentation media was noticed, as well as high correlation between peptide profiles determined using HPLC and the e-tongue output. These results demonstrated that the e-tongue system is a promising tool for fermentation process monitoring and quantitative analysis of growth media (Esbensen et al., 2004).

A hybrid electronic tongue based on a combination of potentiometry, voltammetry, and conductivity was used for classification of six different types of fermented milk (yogurt, kefir, etc.; Winquist et al., 2000). Using ion-selective electrodes, pH, carbon dioxide, and chloride ion concentrations were measured. The voltammetric electronic tongue consisted of six working electrodes of different metals and an Ag/AgCl reference electrode. The various nature of the microorganisms in the different fermentations was reflected on PCA plots both for potentiometric and voltammetric measurements (Fig. 21.4). It was expected that the combination of information obtained from these various techniques, that is, the realization of hybrid e-tongue, should result in a better description of the sample and this effect was observed, then the hybrid tongue could separate all six samples (Fig. 21.4; Winquist et al., 2000).

Classification of six categories of set yogurt was a task for the voltammetric e-tongue comprised of four working electrodes. Various potential waveforms (multi-frequency hackle pulse voltammetry—MHPV, MRPV, MSPV) were applied (Table 21.1; Wei et al., 2013a). The total areas under the corresponding curves obtained in three frequencies were treated as the characteristic values processed by PCA and discriminant function analysis (DFA) for category classification. Voltammetric e-tongue based on MSPV combined with PCA presented the best separation ability in classifying the six categories of yogurt. Satisfactory recognition capability was confirmed by the application of PLS-discriminant analysis (PLS-DA) for category prediction. Additionally, good correlation between physical properties of yogurt samples such as surface stress and viscidity and e-tongue output was observed (Wei et al., 2013a).

Finally, for monitoring of taste and important components changes in probiotic fermented milk during storage, the α-Astree e-tongue was applied (Hruškar et al., 2010a,b; described in Section 21.2). The same device was applied for classification of five brands of yogurt (Hruškar et al., 2009).

## 21.5 ESTIMATION OF FAT CONTENT

The fat content of milk is the amount of milk made up by butterfat—if fat content is higher, the milk has more nutritional energy per cup. To reduce the fat content of milk, usually all of the fat is removed and then the required quantity returned. Fat content can also be altered by selective breeding and genetic modification of cows. For determination of fat content in milk, various tests can be applied, for example, Babcock test. However, it can be also determined/estimated by means of e-tongues, which would be especially desirable in the case of process monitoring as an easy-to-implement method with online control. For such purposes, the flow-through e-tongue based on potentiometric solid-state electrodes was developed (Ciosek et al., 2006a). Their signals formed an input to the SVM neural network without a preprocessing stage. The results of the classification of milk by brand and by fat content proved the proposed system to be very efficient; therefore, the method could find applications in the food industry for checking the parameters of the produced milk, in quality control in dairies, and for the monitoring of milk preparation (Ciosek et al., 2006a).

The disposable, integrated e-tongue for estimation of fat content was constructed with gold CD-R and copper sheets substrates (Paixão and Bertotti, 2009). The sensing elements were gold, copper, and gold surface modified with a layer of Prussian blue. The separated clusters obtained on a PCA plot indicated that milk samples could be clearly differentiated. One of the possible mechanisms to explain this discrimination is associated with different levels of adsorption on the surface of the working electrodes caused by changes in fat content of the milk samples (Paixão and Bertotti, 2009).

Discrimination between milks of various fat content was also possible with the use of SH-SAW-based e-tongue (described in Section 21.3). Moreover, this device was capable to estimate fat content in the range 0.1–4.0% (Cole et al., 2002). The same device was coupled with an electronic nose based on ChemFET sensors; by combining two types of microsensors, an artificial flavor sensing system was developed. Initial tests conducted with milk of different fat content resulted in 100% discrimination using PCA (Cole et al., 2011).

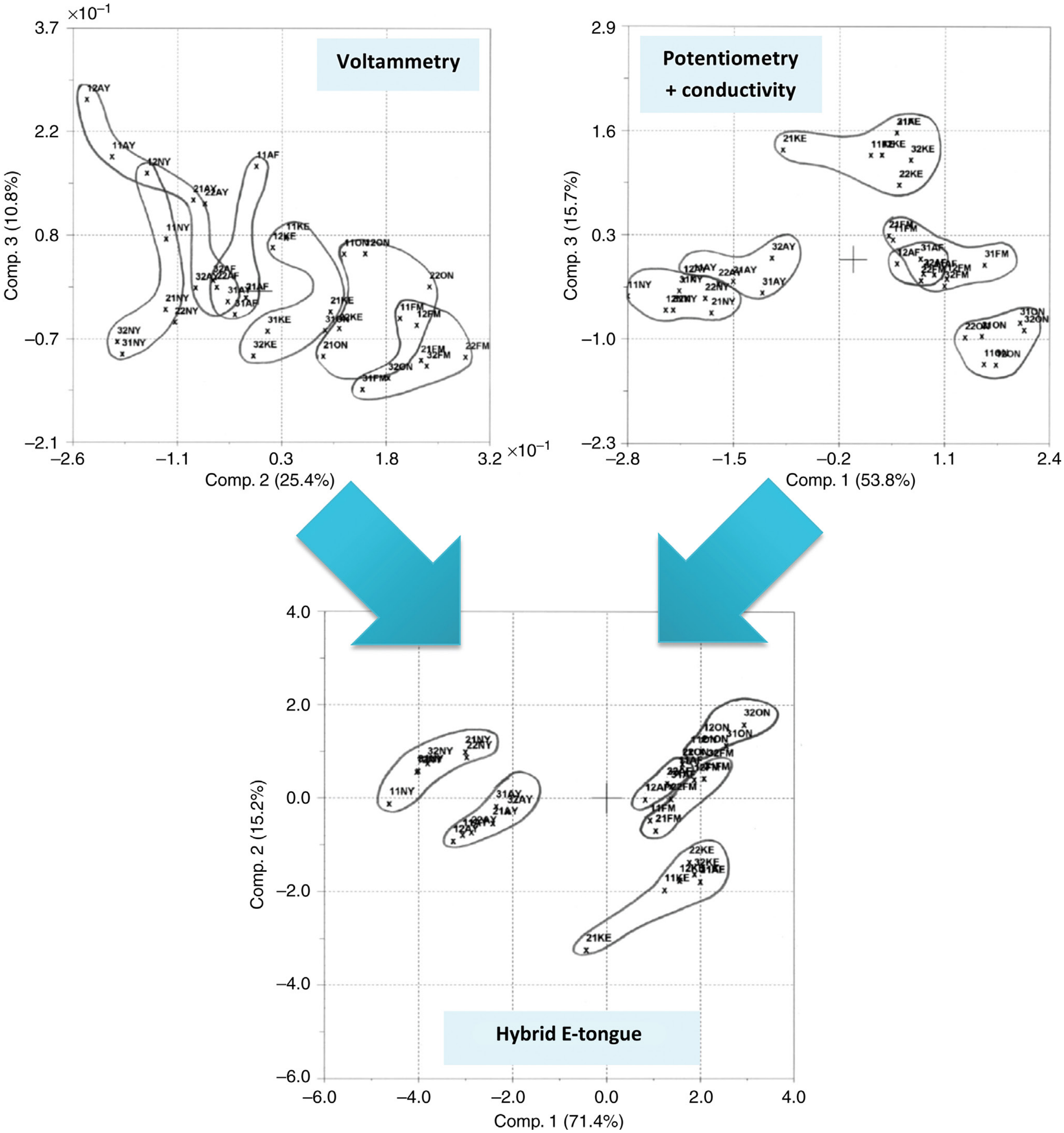

**FIGURE 21.4 Combination of voltammetric, potentiometric, and conductivity measurements in hybrid electronic tongue for better discrimination of fermented milk products.** *(Adapted from Winquist et al., 2000.)*

## 21.6 CLASSIFICATION ACCORDING TO DAIRY ORIGIN/BRAND

Classification of milks according to its origin, and therefore the recognition of its characteristic properties related with processing and taste, serves as a model task in the performance check during the development of new architectures of e-tongue sensor arrays and new numerical procedures for e-tongue pattern recognition units. A potentiometric e-tongue based on selective and partially selective ISEs was applied to qualitative analysis of various brands of milk—the tests of the system were performed using products of the same brand, but with different manufacture dates (and thus comparable in terms of taste). This procedure also allowed to avoid overfitting of the classification model and to capture the true data structure. The sensor array's responses were processed by PCA and ANN, which resulted in very high classification correctness. A sufficient database for beverage recognition was constructed by samples from only two different manufacturer lots. In this way, the real working conditions of the e-tongue were evaluated (Ciosek et al., 2004; Ciosek and Wróblewski, 2007b). The same types of chemosensitive membranes were applied in an array of miniaturized potentiometric sensors—solid-state electrodes working in flow-through mode (Fig. 21.1). Again, the fusion of ion-selective and partially selective microelectrodes with PCA and ANN processing of sensor responses led to the recognition of brands of milks with high correctness (Ciosek and Wróblewski, 2007b; Ciosek et al., 2006b).

New construction of a potentiometric e-tongue for the classification of milks originating from various producers was based on an integrated array of microelectrodes fabricated from epoxy-glass laminate (Fig. 21.1; Ciosek and Wróblewski, 2008). Also in this case, PVC membranes with various additives were used as chemosensitive layers to obtain selective and partially selective sensors. The developed sensor array coupled with PLS-DA was capable of recognizing milk samples with high correctness. Moreover, the application of miniaturized reference electrode, based on ionic liquid and integrated on the same substrate, also provided satisfactory results, which could be helpful in the future construction of handheld electronic tongue systems (Ciosek and Wróblewski, 2008).

Another potentiometric system—the α-Astree e-tongue—was also evaluated by checking its discrimination ability in the recognition of milk samples from different producers. The outputs of ISFETs forming sensor array were processed with the use of PCA resulting in satisfactory discrimination between five brands of milk (Hruškar et al., 2009). Combined e-tongue and e-nose device (described in Section 21.3) based on a voltammetric cell with four working electrodes and eight MOS sensors, was applied to the discrimination between five milk brands. 3D PCA plots revealed appropriate recognition ability of this system (Bougrini et al., 2014).

Classification accuracy of e-tongues can be significantly improved using appropriate numerical methods for the analysis of sensor array responses. This effect was studied extensively and the results were presented in various papers (Ciosek et al., 2006a,c; Ciosek and Wróblewski, 2006). Potentiometric sensor array responses were processed with the use of various linear and nonlinear procedures: SVM (Ciosek et al., 2006a), *k*-nearest neighbors—*k*-NN (Ciosek and Wróblewski, 2006; Ciosek et al., 2006c), PLS (Ciosek and Wróblewski, 2006), soft independent modeling of class analogy—SIMCA (Ciosek and Wróblewski, 2006), back propagation neural network—BPNN (Ciosek and Wróblewski, 2006; Ciosek et al., 2006c), learning vector quantization—LVQ (Ciosek and Wróblewski, 2006), probabilistic neural network—PNN (Ciosek et al., 2006c). Classification accuracy in the model task of milk brand recognition was compared and some analogies with general rules referring to electronic nose were found. LVQ networks were proved to exhibit the best performance. Their further advantages, such as fast training and robustness, make them the suggested pattern classifiers for sensor array data (Ciosek and Wróblewski, 2006).

## 21.7 INDUSTRY-ORIENTED APPLICATIONS

Due to their ruggedness and simplicity, electrochemical e-tongues are especially suitable for online monitoring of industrial processes. The first voltammetric e-tongue, specially designed for use in the dairy industry, was presented in 2005 (Winquist et al., 2005). It consisted of four working electrodes made of various metals embedded in PEEK™. It was mounted in a housing of stainless steel, which was inserted in the process line for direct online measurements. There are a number of flavor categories of milk such as sour, salty, fishy (trimethylamine), blueberry, unclean, oxidized, rancid, and chemical; in addition, there can be taste from the cattle's food such as ensilage, clover, and hay, which has a seasonal variation. There is always a risk that milk from one farm can contaminate part or all of the shipment transported to the dairy plant in a single tank truck. An even greater risk is if off-flavor milk enters a storage silo in the dairy plant and contaminates a large volume of raw milk. Therefore, the voltammetric e-tongue was used to follow different sources of milk coming into the process and to monitor the cleaning process. The results showed that milk from different sources, and thus also having different quality properties, could be distinguished. This opens up the possibility for monitoring off-flavors in the incoming milk and it makes e-tongue a valuable security tool to prevent economic loss (Winquist et al., 2005).

The adulteration of goat milk with bovine milk is quite frequent, due to the seasonal fluctuations of the production of goat milk and to its higher price. Therefore, it is important to establish and validate easy and reliable methodologies that can be used to detect this kind of adulterations. Although various methods such as HPLC, immunological assays, and immunochromatography were proposed in recent years for the detection and/or quantification of milk and cheese adulterations, they are very time consuming and expensive, requiring complex pretreatment of the samples, specialized equipment, and qualified personal. Therefore, the development of an e-tongue that could be used in the dairy industry by cheese makers to evaluate in a simpler, faster, more economical way and in real time the possible adulterations of their "raw material" is of major importance. An array of cross-sensitive solid contact electrodes was applied to the detection of goat milk adulteration with bovine milk (Dias et al., 2009). The applied linear discriminant analysis (LDA) model could distinguish between raw skim milk groups (goat, cow, and goat/cow) with an overall sensitivity and specificity of 97 and 93%, respectively. Furthermore, cross-validation showed that the model was able to correctly classify unknown milk samples with a sensitivity and specificity of 87 and 70%, respectively (Dias et al., 2009). Detection of adulteration of milk with hydrogen peroxide was possible with the use of a disposable voltammetric electronic tongue (Paixão and Bertotti, 2009; described in Section 21.5).

The quality of milk introduced to the dairy plant is also checked from a microbiological point of view. Bovine mastitis is the inflammation of the bovine mammary gland, caused by pathogen infection. This disease is one of the largest production concerns in the dairy industry, due to high cost associated with lost yield, discarded milk, cost of veterinarian treatment, the herdsman's time, and extended calving intervals. Mastitis milk contains both pathogens and bacterial toxins; therefore, its consumption increases the risk of ingestion and transmission of pathogens and ingestion of toxins. Thus, the detection of the clinical infectious disease is a legal requirement for milk for human consumption in most developed countries. In robotic milking systems, mastitis detection is currently performed by a combination of human inspection of animals, by electrical conductivity, and by analysis of changes in milk yield. However, still early warning is not reliable with sensors and software currently on the market. As an alternative, an e-tongue based on an ISE sensor array was proposed for the discrimination between milk secretions from infected and healthy glands (Mottram et al., 2007). It was demonstrated that the multisensor system could distinguish between control and clinically determined mastitis milk samples. The sensitivity and specificity of the sensor system (93 and 96%, correspondingly) showed an improvement over conductivity (56 and 82%, correspondingly).

Antimicrobial agents are routinely administered to food-producing animals to promote growth as well as for therapeutic and prophylactic reasons. However, the passage of antibiotics into milk from medicated animals influences the quality of raw milk and such residues constitute a potential risk to the consumer, causing allergies and creating possible resistance of microorganisms to the introduced antibiotics (Wei and Wang, 2011). Moreover, the residues can lead to considerable losses in fermented products such as cheese or yogurt (eg, they inhibit the bacterial fermentation processes). A voltammetric e-tongue was developed to detect six antibiotic residues in bovine milk: chloramphenicol, erythromycin, kanamycin sulfate, neomycin sulfate, streptomycin sulfate, and tetracycline HCl. The samples contained spiked residues at four concentration levels: 0.5, 1, 1.5, and 2 maximum residue limits. They were classified on the basis of e-tongue responses processed with the use of PCA and DFA. Three regression models—principal components regression (PCR), PLS, and LS-SVM—were used for the prediction of antibiotics concentrations. All the regression models performed well, and PCR had the most stable results (Wei and Wang, 2011).

Various byproducts of the dairy industry, such as whey or whey proteins, are used as additives in many processed foods, including breads, crackers, commercial pastry, and animal feed. Whey protein is often sold as a nutritional supplement and can be applied as a fertilizer or flour conditioner. Whey can be also applied as a substrate in methane fermentation. During this biotechnological process, also known as anaerobic digestion, waste is transformed by microorganisms under anaerobic conditions and valuable biogas is produced. The process itself, its duration, and the quantity of biogas achieved depend on the raw material and bacteria used as an inoculum. There are many factors that can disturb the process itself, or slow it down (eg, an increase in volatile fatty acids and a decrease in the pH of bulk solution subsequently inhibits the methanogensis step and leads to process failure). The interdependence of the different microbial groups involved in anaerobic digestion is the main cause of the process instability. Therefore, there is a need to monitor fermentation not only by pH measurement or biogas analysis, but also by more detailed observation of the liquid phase in the bioreactor. For such a purpose, flow-through arrays of potentiometric electrodes were applied (solid-state planar electrodes or miniaturized ISE with classical architecture; Buczkowska et al., 2010; Witkowska et al., 2010). Whey fermentation was observed by studying two main factors—volatile fatty acid (VFA) content and chemical oxygen demand (COD)—that were determined with the use of classical off-line methods. The monitoring performed by e-tongue allowed for satisfactory determination of VFA level and COD, and thus evaluation of fermentation stage (Fig. 21.5). The applied flow-through sensor array is applicable for online process control, and its modular architecture is advantageous when composing a sensor set and regarding its future applications (Buczkowska et al., 2010; Witkowska et al., 2010).

Another example of the possible use of e-tongues in the dairy industry is the analysis of dairy by-products and protein hydrolysates. The taste of bovine milk whey protein produced as a by-product from cheese and butter manufacturing was studied with the use of a taste sensor (Sano et al., 2005). The taste of dairy protein hydrolysates was estimated by α-Astree e-tongue (Newman et al., 2014a, 2014b; both applications described in Section 21.2).

## 21.8 CONCLUSIONS

In this chapter, various aspects and possibilities of using e-tongue methodology to milk and dairy products analysis were presented and discussed. It was shown that the multisensor

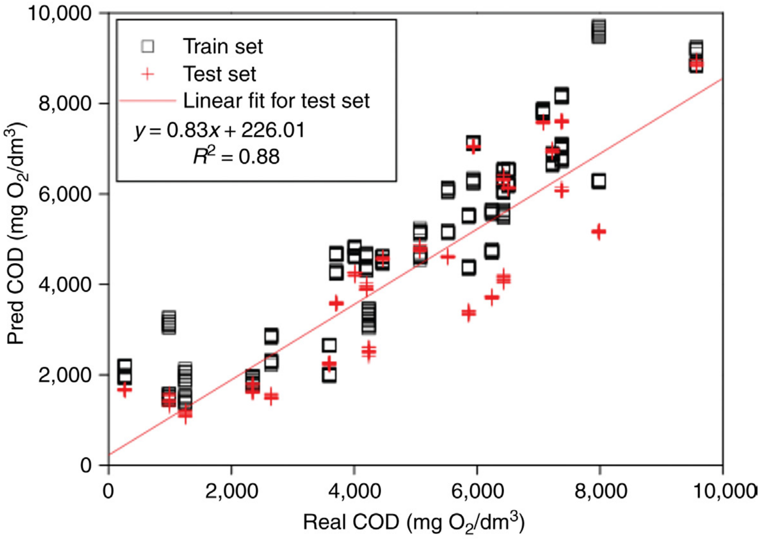

**FIGURE 21.5 PLS prediction of chemical oxygen demand (COD) for whey samples in methane fermentation process obtained by potentiometric e-tongue based on modular flow-through sensor array.** *(Adapted from Witkowska et al., 2010.)*

approach allows for wide adaptability of such systems to various qualitative/semiquantitative/quantitative tasks, even when such challenging samples (with high protein content) are analyzed. Simplicity of measurements, the low cost of analysis, and the possibility to perform the analysis in real time and online make e-tongues a very attractive alternative to traditionally used techniques. Two e-tongue systems commercially available, such as the taste sensing system and the α-Astree e-tongue, are usually applied to taste sensing of milk and classification/recognition of various milk products. Most of studies presented in this chapter employing prototype versions of e-tongues indicate for much wider area of applications: freshness evaluation, microbial growth monitoring, quality control studies, and detection of adulterations. Moreover, last ten years evidenced that there is a trend to elaborate e-tongues for industry applications, especially process monitoring. For such purposes, many research on the coupling of e-tongues with distributed expert systems for the advanced in-line monitoring of food production processes, and the application of various flow-based e-tongues will be probably conducted in the next few years.

Nevertheless, it must be remarked that the main challenge now is the production of sensor arrays with very repeatable electrochemical properties, minor ageing effects, as well as minimization of irreversible binding of substances to chemosensitive layers of sensors, which leads to some calibration instability. Other important missing aspects are the lack of long-term studies, sometimes nonreliable validation that influences the estimation of predictive ability of the developed systems, and almost none interlaboratory studies (Ciosek and Wróblewski, 2007a; Riul et al., 2010; Peris and Escuder-Gilabert, 2013; Escuder-Gilabert and Peris, 2010). However, there is no doubt that e-tongues may be used for the resolution of various complex analytical problems and since this is quite a new methodology, following development of such systems not only for milk and dairy products analysis is expected.

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## Chapter 22

# Monitoring of Fermentation and Biotechnological Processes

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## 22.1 INTRODUCTION

Fermented foods are "those foods that have been subjected to the action of micro organisms or enzymes so that desirable biochemical changes cause significant modification in the food" (Campbell-Platt, 1994). Fermentation is a conversion of complex organic substances, mostly carbohydrates, into simpler compounds by the action of microorganisms such as bacteria, yeasts, or molds. From the chemical perspective, fermentation consists of a series of reactions catalyzed with enzymes in the living cells. Fermentation is one of the oldest methods of the food processing and preservation, increasing the nutritious value of food, making it more digestible, making it tastier, or preventing its spoilage, or all of them. The most widespread examples have been the use of lactic acid bacteria for production of cheese and other dairy and yeasts for alcoholic fermentations and bread (Ross et al., 2002). For millennia, fermented food was artisanal small-scale products relying on the experience handed down the generations. Modern fermented food manufacturing, with some exceptions such as cocoa fermentations, is as sophisticated and controlled as any industry.

The active compounds of all fermentation processes, the enzymes, are very sensitive to environmental conditions. Even a small uncontrolled change in composition, pH, temperature, and pressure can alter cell metabolism and change radically process efficiency and productivity and can even render the process unprofitable. Being a typical biotechnological process, fermentation is susceptible to all the issues common to this field: poor batch-to-batch reproducibility, unexpected process deviations, inconstant end-product quality. These problems can be minimized or avoided through establishment of appropriate chemical monitoring of a process that can provide for well-timed operation decisions. However, analytical tools for the online process control remain relatively primitive until now. It is worth noting that lack of analytical instruments is a common problem for all biotechnological industries, despite the fact that products worth billions of dollars are produced through fermentation processes annually. Lack of online sensors for the monitoring of the fermentation processes is commonly stressed in the literature (Vojinovic et al., 2006; Clementschitsch and Bayer, 2006). The only chemical parameters being routinely measured in the bioreactors are pH, redox potential, and $pO_2$ (Schugerl, 2001; Harms et al., 2002). Although these parameters are crucial for the fermentation process, they are not always sufficient for effective process control and quite often are not related to the quality of the end product. The lack of chemical information about the process may lead to the fermentation being run under suboptimal conditions, batch contamination, and so forth, all of which result in the unacceptable end-product quality and rather heavy economical losses. Timely identification of the problems would allow traditional laboratory analysis in offline mode in spite of being rather informative takes a lot of time due to various sample pretreatment procedures and involvement of complex analytical equipment.

Several analytical instruments have been proposed for online monitoring of the fermentation processes, the most important of which are near-infrared (NIR) spectroscopy and image analysis (Vojinovic et al., 2006; Schugerl, 2001; Scarff et al., 2006; Sonnleitner, 2013). Both techniques are nondestructive and rapid and require no sample preparation, which makes them quite attractive for the real-time process follow-up. Being noncontact techniques, both NIR and image analysis are devoid of such problems as contamination of the probe by the broth compounds and sterilization. NIR spectroscopy is currently widely used in agricultural, food, and pharmaceutical industries as a quality control tool given that problems with instrumental drift and calibration stability have been solved. Artificial vision or image analysis makes use of images collected using a charge-coupled device (CCD) or other type of camera. This is a new technique that has not yet been widely applied in the industry.

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Both NIR spectroscopy and image analysis require relatively complex statistical methods for the data processing and modeling, especially in the case of image analysis. It should be noted that wider application of NIR in the biotechnological industry is impeded by the complexity of analyzed media and the fact that only relatively high concentrations can be quantified using this technique.

Another analytical instrument, which is particularly suitable for the tasks of online or at-line process monitoring, is an electronic tongue. According to the IUPAC definition, "The electronic tongue is an analytical instrument comprising an array of nonspecific, low-selective, chemical sensors with high stability and cross-sensitivity to different species in solution, and an appropriate method of pattern recognition and/or multivariate calibration for data processing" (Vlasov et al., 2005). The electronic tongue shares advantages of the chemical sensors such as the possibility to perform measurements in real-time, easy automation of measurements and the relative simplicity and low price of the required instrumentation. Besides, the electronic tongues allow overcoming some of the chemical sensors' limitations such as insufficient selectivity in the multicomponent media.

The purpose of this chapter is to review the review applications of electronic tongues to the monitoring of food fermentation processes.

## 22.2 REQUIREMENTS FOR THE SENSORS FOR BIOPROCESS MONITORING

The main requirements for the sensors to be applicable to the fermentation monitoring can be summarized as follows. They should be able to provide information about concentrations/parameters of interest in real time and do not compromise sterility of the process. Different approaches may be implemented with the aim to obtain real-time information about the fermentation process. Sensors can be placed inside the bioreactor (in situ sensors) or a sample can be taken from the bioreactor and analyzed in the close proximity to the process. In situ sensors appear to be the more attractive option, which is, however, the most difficult to implement in practice. In situ sensors should be capable of functioning during prolonged periods of time (at least for the duration of the process) without external interventions. That is, sensors should not suffer from fouling of its surface by the biomaterial, primarily proteins, and should not require recalibration for the duration of the bioprocess. Furthermore, sensors must endure rather extreme conditions during the sterilization without deterioration of its characteristics. While the latter concern has been addressed and several sterilizable probes can be found on the market, fouling and baseline drift remain problematic. Not surprisingly, a very limited number of in situ chemical sensors are currently used for fermentation monitoring, mainly already mentioned pH, redox, and $O_2$ probes (Schugerl, 2001; Harms et al., 2002).

At-line or online sensors appear to be more feasible solutions as they allow to carry out cleaning, conditioning, and recalibration of the sensors, when necessary, and eliminate the need for sterilization. Implementation of sensors in flow-injection (FIA) or sequential-injection (SIA) setup permits to automate all analytical steps including cleaning, calibration, and measurements. Due to their low response time, the high sampling frequency, versatility, and flexibility, the FIA and SIA systems established them as a key tool for bioprocess monitoring (Harms et al., 2002).

Furthermore, sensors should be able to measure substances of interest in the range of their variation during the process that is to have adequate dynamic ranges and detection limits. Sensors should possess sufficient selectivity toward measured substances to be capable of carrying out determination in the complex environment such as fermentation broth where several other compounds are present often in high concentrations. The latter represent the biggest challenge as most chemical sensors, as well as other types of probes such as NIR spectroscopy, suffer from insufficient selectivity in multicomponent media. This led to the introduction of the "soft sensors" concept also referred to as "software" or "virtual" sensors as a possible solution (Luttmann et al., 2012). "Soft sensors" are defined as a combination of relatively nonspecific but accessible measuring tool with sensor signal evaluation models. In practice, "soft sensors" allow us to use easily accessible online data for the estimation of other variables that are otherwise either difficult to measure or only measured at low frequency. Alternatively, soft sensors can also be used with the purpose of performing fault detection and diagnosis of the bioprocess. In the field of chemical sensors and biosensors, "soft sensors" are called electronic tongues, the definition of both being very similar (Vlasov et al., 2005).

Sensors based on a variety of transduction principles are used in the electronic tongue systems, including electrochemical (potentiometric, amperometric, impedimetric), gravimetric (surface-acoustic wave, quartz crystal microbalance, etc.), and optical (fluorescence, etc.) (Legin et al., 2003; del Valle, 2010; Rudnitskaya and Legin, 2008). For the fermentation monitoring, only potentiometric, optical, and hybrid combining potentiometric with amperometric electronic tongues have been employed. Types of chemical sensors used in the electronic tongue systems together with detected analytes and process parameters are listed in Table 22.1.

## 22.3 MODEL FERMENTATION SOLUTIONS

When dealing with analysis of complex multicomponent media, such as different biotechnological solutions, it makes sense to approach the problem by studying model solutions first. In this way, appropriate measuring procedures can be established and possible interferences for the

**TABLE 22.1** Sensors for the Electronic Tongues and Electronic Noses

| Transduction Mode | Sensing Materials | Analytes/Parameters | Fermentation Process |
|---|---|---|---|
| Potentiometric | Plasticized organic polymers modified by ionophores, chalcogenide glasses | Organic acids, ammonia | Citric acid production by *A. niger*, model solutions (Legin et al., 2004) |
| | | Acetic acid, optical density | Model *E. coli* fermentation (Turner et al., 2003) |
| | | Octanoic acid | 2-Heptanone production by *P. roqueforti* (Lomborg et al., 2008) |
| | | Recognition of microorganisms, growth stages | Model fermentation of the fungi, yeasts, and bacteria (Soderstrom et al., 2005a) |
| | | Organic acid, deviation of the process from normal operating conditions | Fermentation of the starting culture for the cheese production (Esbensen et al., 2004) |
| | | Amino acids, total acidity | Miso production (Imamura et al., 1996) |
| | | Ethanol, titratable acidity | Sake production (Arikawa et al., 1996) |
| | | Titratable acidity, fermentation time | Kimchi maturation (Kim et al., 2005) |
| Voltammetric | Noble metals (Au, Ir, Pt, and Rh) | Ergosterol; recognition of microorganisms, growth stages | Model fermentation of the fungi, yeasts, and bacteria (Soderstrom et al., 2003a,b; Soderstrom et al., 2005a,b) |
| Hybrid | Potentiometric (plasticized PVC, redox) + voltammetric (glass carbon) | Total acidity, process duration prediction, contamination identification | Citric acid production by *A. niger* (Kutyła-Olesiuk et al., 2014) |
| | | Process stages recognition, process duration prediction | Beer fermentation (Kutyła-Olesiuk et al., 2014) |
| | Potentiometric (plasticized PVC, chalcogenide glasses) + voltammetric (noble metals) | Recognition of microorganisms, growth stages | Model fermentation of the fungi, yeasts, and bacteria (Soderstrom et al., 2005a) |
| Optical | SPR | Discrimination of different fermentation times | Sake fermentation (Nanto et al., 1999) |
| | | | Vinegar production (Nanto et al., 2002) |

analytes and parameters of interest can be clarified. Application of potentiometric electronic tongue to the analysis of model solutions closely mimicking the composition of the broths from the fermentation with filamentous fungi *Aspergillus niger* was described in Legin et al. (2004). *A. niger* is widely employed in food biotechnology, in particular in the production of citric acid (Grewal and Kalra, 1995). During the fermentation process, fungi consume glucose and ammonium from the culture media to produce organic acids, such as citric, oxalic, pyruvic, and some other substances such as polyols. The electronic tongue was applied for potentiometric measurements in 22 model solutions whose compositions were following the course of typical fermentation in time. The data for modeling were obtained from the measurements in real fermentation broth samples performed with standard analytical techniques such as high-performance liquid chromatography (HPLC). Solutions contained a constant background of inorganic salts such as potassium chloride and dihydrogen phosphate, magnesium sulfate, and trace elements, whereas the content of citrate, pyruvate, oxalate, glucose, glycerol, mannitol, erythritol, and ammonium chloride was changed in a relevant range to mimic the progress of fermentation. Moreover the influence of a sodium azide addition on sensor response was also investigated. This substance is normally added to the broth to stop the microbial activity and to "freeze" chemical composition of the media for analysis. It was shown that a potentiometric electronic tongue can provide for simultaneous quantitative assessment of ammonium, oxalate, and citrate content in simulated fermentation media of *A. niger*. The studied concentration ranges of the components were 0.4–14 mM for ammonium, 0.5–5.5 mM for citrate, and 2.6–62.2 mM for oxalate. Mean relative errors of prediction of these components were 6, 6, and 8%, correspondingly. Data processing was done using two different regression techniques: partial least squares (PLS) and back propagation artificial neural networks (BP–ANN), the latter provided for higher precision since it can effectively deal with nonlinear

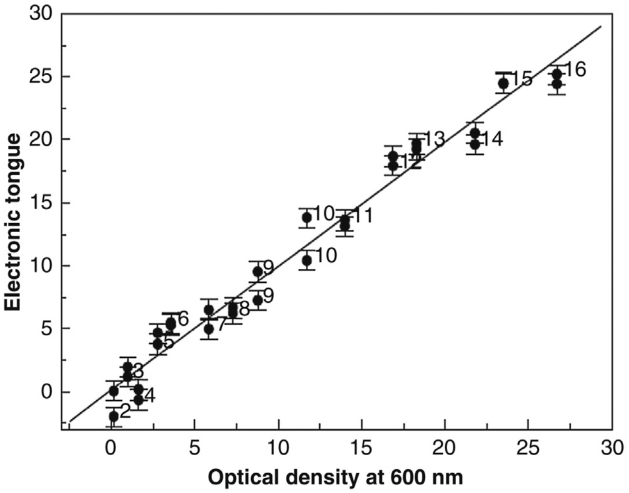

**FIGURE 22.1 Estimation of optical density in fermentation media from the electronic tongue response.** *(Reprinted from Turner et al., 2003, with permission from Elsevier.)*

sensor response induced by high concentration of interfering ions. The content of all three components of interest can also be quantified in the presence of 10 mM sodium azide.

Noteworthy, later on it was shown that analysis of real fermentation samples from citric acid production with *A. niger* is also fully possible by means of an electronic tongue system consisting of potentiometric and voltammetric sensors (Kutyła-Olesiuk et al., 2014). The measurements were performed in offline mode.

Another example of dealing with model fermentations was provided in Turner et al. (2003). In this study, the potentiometric electronic tongue system was applied for quantification of dry weight content, optical density, and acetic acid in broth samples taken from the model fermentation process (not intended for real production of some substances) with *Escherichia coli*. Recombinant strains of these bacteria are widely employed, for example, for synthesis of heterologous (foreign) proteins for pharmaceutical industry. With the help of the potentiometric multisensory system of 21 sensors, it was possible to track the fermentation progress in the broth sampled with certain time intervals in the course of 10-h-long fermentation. Particular attention was paid to the analysis of acetic acid content, which is an unwanted byproduct of glucose metabolism in bacteria and increased levels of this substance can lead to inhibition of protein production in the recombinant strain. Reference data on acetic acid concentration were obtained from HPLC measurements. The mean relative error (MRE) in prediction of acetate from electronic tongue response was 11%. Taking into account the time of analysis with multisensor system (3 min instead of the time-consuming HPLC procedures), this can be considered a promising result. Another interesting outcome of the study is the observed correlation between the optical density and the electronic tongue response (Fig. 22.1). Optical density is an important indicator of biomass growth in the media. MRE for optical density analysis was 13%. Obviously optical density of the fermentation broth depends a lot on its chemical composition and this is why such correlation can be established, thus converting potentiometric sensors into the "optical" instrument. The study also reports on the possibility of dry weight estimation in fermentation samples with an electronic tongue (MRE 16%).

Various fungi and yeast species may be employed in the fermentation processes, but they also may act as contaminants of the fermentation cultures and foods. Thus, a generic experiment looking at the capability of the electronic tongue system to distinguish different species grown in the same conditions is of interest. A series of works reported applications of the voltammetric and potentiometric electronic tongues to the recognition and growth monitoring of microbial species including fungi, yeasts, and bacteria. Voltammetric electronic tongue comprising four noble metal working electrodes (gold, iridium, platinum, and rhodium) was used for the monitoring of the growth of *Aspergillus oryzae* and quantification of ergosterol, compounds produced exclusively by fungi (Soderstrom et al., 2003a). Measurements were carried out in the three electrode scheme using differential pulse voltammetry. The electronic tongue was capable of detecting mold growth after ca. 40 h after inoculation and has shown good correlation with ergosterol content in fermentation broths. The same setup was applied to the recognitions of six microbial species (three fungi, one yeast, and two bacteria) grown at the same conditions (Soderstrom et al., 2003b). They could be differentiated by the electronic tongue after the logarithmic growth stage is reached, which was ca. after 30 h after inoculation. The same voltammetric setup and potentiometric

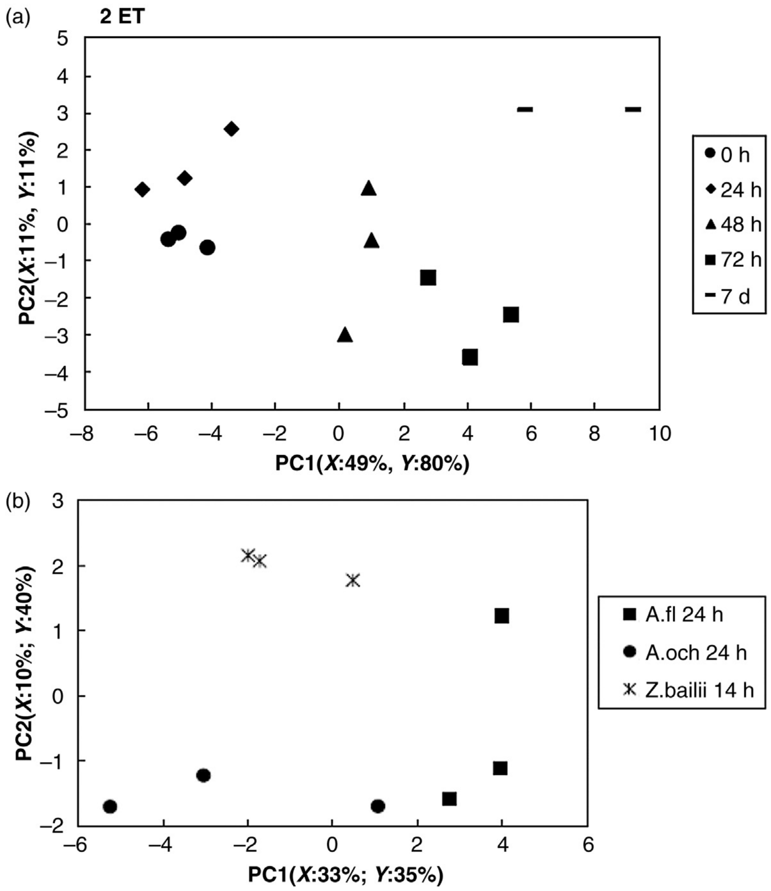

**FIGURE 22.2** (a) Discrimination of *A. ochraceus* broths sampled at different growth stages (0, 24, 48, and 72 h and 7 days) and (b) discrimination of broths of two molds (*A. ochraceus* and *A. flavus*) and yeast (*Z. bailii*) sampled in the beginning of the log growth phase, that is, after 24 and 14 h of inoculation, correspondingly. The PLS score plot was produced using merged data from two electronic tongues. *(Reprinted from Soderstrom et al., 2005a, with permission from Elsevier.)*

electronic tongue comprising 20 sensors with plasticized PVC and chalcogenide glass membranes was applied to the recognition and growth monitoring of the four *Aspergillus* species (*A. flavus*, *A. oryzae*, *A. ochraceus*, and *A. versicolor*) and the yeast *Zygosaccharomyces bailii* (Soderstrom et al., 2005a). Both electronic tongues were capable to distinguish cultures grown to the stationary stage (7 days for molds and 4 days for yeast), except *A. flavus* and *A. oryzae*. Both electronic tongues could follow the growth of two molds (*A. flavus and A. ochraceus*) and the yeast. Voltammetric electronic tongue was found to be more sensitive to the later growth stages being capable to discriminate broth sampled 48 h and 27 h after inoculation for mold and yeast, respectively. The potentiometric electronic tongue was more sensitive to the changes in the broth occurring in the beginning fermentation being capable to recognize samples taken before inoculation and in the beginning of log growth phase, that is, sampled 24 and 48 h after inoculation for molds and 14 and 27 h for yeast. Superior performance was obtained after merging of the data from two devices, which allowed to differentiate broths sampled during the entire growth process (Fig. 22.2a) as well as discriminate three species at the beginning of the stationary growth phase (Fig. 22.2b).

Recognition of *A. flavus* and *Penicillium commune* grown in different media (malt, sucrose, and glucose) as well as monitoring of their growth were carried, using the same setup of the voltammetric electronic tongue (Soderstrom et al., 2005b). Similarly to the results previously described, the electronic tongue was capable of discriminating broths at the later stages of growth, in this case, 7 and 10 days after inoculation, while samples taken before 1 and 3 days after inoculation could not be distinguished.

## 22.4 FOOD ADDITIVES

In the last decades biotechnological advancements have gained importance in the production of food additives and flavorings. The number of compounds produced using biotechnology is increasing exponentially, replacing such processes of the manufacturing of food additives as extractions from the natural sources and chemical synthesis.

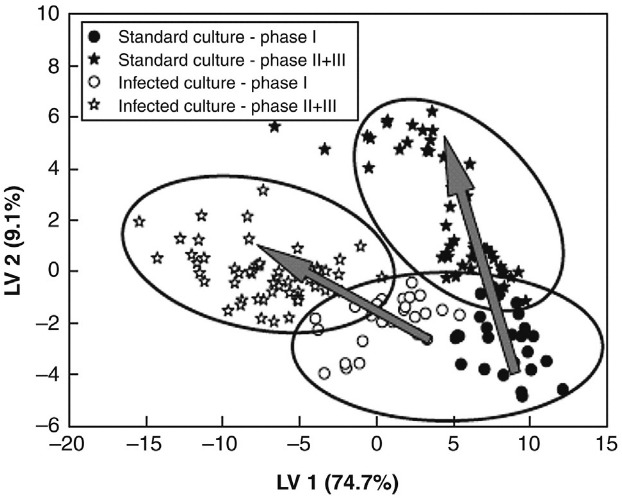

**FIGURE 22.3 The PLS-DA plot of chemical images of the samples collected during the standard and infected fermentation process (data obtained using hybrid ET).** *(Reprinted from Kutyła-Olesiuk et al., 2014, with permission from Elsevier.)*

Biotechnology has the advantages of being less expensive than extraction and producing compounds that are closer to the natural counterparts (ie, the same enantiomers as encountered in nature) than chemical synthesis. Biotechnologically produced compounds include food pigments (phycocyanin, lutein, β-carotene, astaxantin, etc.), polyunsaturated fatty acids (eicosapentaenoic acid, docosahexaenoic acid, γ-linolenic acid), organic acids (citric, lactic, acetic, tartaric, L-glutamic acid), vitamins, and volatile flavor compounds (Chen, 2004; Schrader, 2007; Gounaris, 2010).

The first industrial fermentation process aimed at the production of the food additive production was production of citric acid by the *A. niger*. It was implemented in the beginning of the 20th century and remains to this day the main commercial source of this acid. Besides citric acid, *A. niger* is also employed in the fermentation processes for production of other organic acids and enzymes, hence the interest for the monitoring of this fungi fermentation. Quantitative analysis of the model solutions imitating broths from *A. niger* fermentation was previously described (Legin et al., 2004). Analysis of the solutions from the real fermentation process of citric acid production by *A. niger* using the hybrid electronic tongue has been reported in Kutyła-Olesiuk et al. (2014). Hybrid electronic tongue comprised 12 potentiometric sensors with plasticized PVC membranes with different ionophores, glass pH, and metallic redox electrode and voltammetric glass carbon electrode. Fermentation follow-up, detection of the culture contamination by the yeast *Saccharomyces cerevisiae* ca. 25 h after, and quantification of total acidity were done (Fig. 22.3). It was observed that for this application, the hybrid electronic tongue performed better compared to only potentiometric or voltammetric sensors alone. Analysis with the electronic tongue was performed offline; that is, fermentation broth was sampled and frozen at −20°C, thawed, and analyzed simultaneously.

An electronic tongue was applied to the detection of octanoic acid in broths from an industrial fed-batch cultivation of *Penicillium roqueforti* for production of “natural” 2-heptanone (Lomborg et al., 2008). 2-Heptanone is an important aroma compound in Roquefort cheese and is therefore used as blue cheese flavoring in the products like salad dressings, soups, and crackers. Biosynthesis of 2-heptanone is the most effective at the intermediate concentrations of the octanoic acid, in the range of 5–10 mM. Thus, close monitoring of octanoic acid catabolism with the aim to maintain its concentration constant is desirable. Electronic tongue comprising six anion-sensitive sensors with plasticized PVC membranes was calibrated using broth samples from four fermentation runs, using octanoic acid concentration determined by HPLC as a reference. Validation of the obtained PLS calibration model using samples from fifth fermentation run has shown that prediction errors were similar for the calibration and validation samples.

## 22.5 FOOD FERMENTATIONS

Various types of fermentations are widely applied in the food industry for production of different food products and drinks. Applications of the electronic tongues to the monitoring of these processes are discussed in this chapter, except alcoholic fermentation, which will be described in Chapter 28 *Alcoholic Fermentation Using Electronic Nose and Electronic Tongue* of this book.

In Imamura et al. (1996), an electronic tongue system based on eight polymeric plasticized membranes was applied for monitoring of miso (soybean paste) fermentation and storage. The response of the sensors was found to be

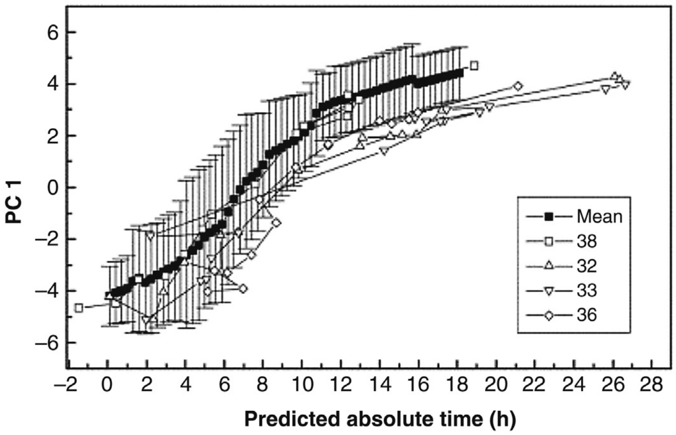

**FIGURE 22.4 Predicted absolute fermentation time for three "abnormal" (32, 33, and 36) fermentation runs and one "normal" (38) run, plotted on the corresponding absolute time control chart.** PLS calibration model based on four "normal" batches was used for producing control chart and predicting fermentation time for test samples. *(Reprinted from Esbensen et al., 2004, with permission from Springer.)*

correlated with content of amino acids and with titratable acidity, which gives a possibility to assess the ripeness of the product. It was also possible to discriminate between three different kinds of miso according to their composition. It is noteworthy that in this research the authors did not apply some dimensionality reduction methods (eg, principal component analysis) and all discussion was done in the original data space of eight sensors.

Two types of the electronic tongues have been applied to the monitoring of sake fermentation. A potentiometric electronic tongue was used for the quantification of ethanol and titratable acidity in sake mash samples (Arikawa et al., 1996). Samples from the brewery fermentation tanks were centrifuged before analysis and the measurements were performed in supernatants. Individual sensors from the array were able to track the growth of the ethanol content through the course of fermentation (19 days). A multiple linear regression model based on responses of two particular sensors of the array was able to predict titratable acidity in sake mash with reasonable precision. An array of four surface plasmon resonance sensors with hydrophobic organic Langmuir–Blodget sensitive layer was employed for the determination of the sake fermentation time (Nanto et al., 1999). The system was capable of distinguishing sake fermented for 5, 14, and 23 days.

The same four sensor system was used to determine the fermentation time of vinegar (Nanto et al., 2002). Discrimination of the vinegar samples fermented for 1, 7, and 14 days was possible.

Fermentation of the starting culture used for cheese production was addressed in the study by Esbensen et al. (2004). Measurements in samples taken from 5 normal and 3 abnormal fermentations were performed with a sensor array comprising 30 potentiometric sensors with polymeric and chalcogenide glass membranes. Using the PLS (projection on latent structure) regression of the sensors' response against elapsed fermentation time, it was possible to produce control charts to judge on the type of analyzed process (normal vs. abnormal) at the early stage. The example of this chart is given in Fig. 22.4. Since abnormal process runs lead to serious economic losses in industry the suggested monitoring approach appears to be very attractive.

The report by Kim et al. (2005) describes the application of sensor array composed by eight polymeric membrane electrodes to follow the ripening of kimchi, a traditional Korean dish made of fermented vegetables. The response of the sensors was strongly correlated with titratable acidity, which increased during kimchi ripening, consequently, with fermentation time (Fig. 22.5).

The report by Kutyła-Olesiuk et al. (2012) describes the application of a hybrid multisensor system comprised of 11 potentiometric microsensors (10 with plasticized PVC membranes and one silicon redox sensor) and voltammetric microsensor with Au working electrode for monitoring of beer fermentation (Ziółkowski et al., 2013). Samples were taken from homemade beer fermentation: fermentation reaction itself and maturation of beer. It was shown that the electronic tongue system can follow both the fermentation and the aging processes. Prediction of the fermentation duration was also possible. The fusion of two types of the data (from potentiometric and from voltammetric sensors) allowed for improvement of the classification capability of the system.

## 22.6 CONCLUSIONS

Biomimetic sensor systems for liquid analysis, such as electronic tongues, represent a novel approach to the application of chemical sensors combining a biologically inspired architecture with latest achievements in the sensor science itself. They possess all the advantages of the chemical sensors such as rapid measurements, possibility of easy automation of the sensor setup, and relatively simple and

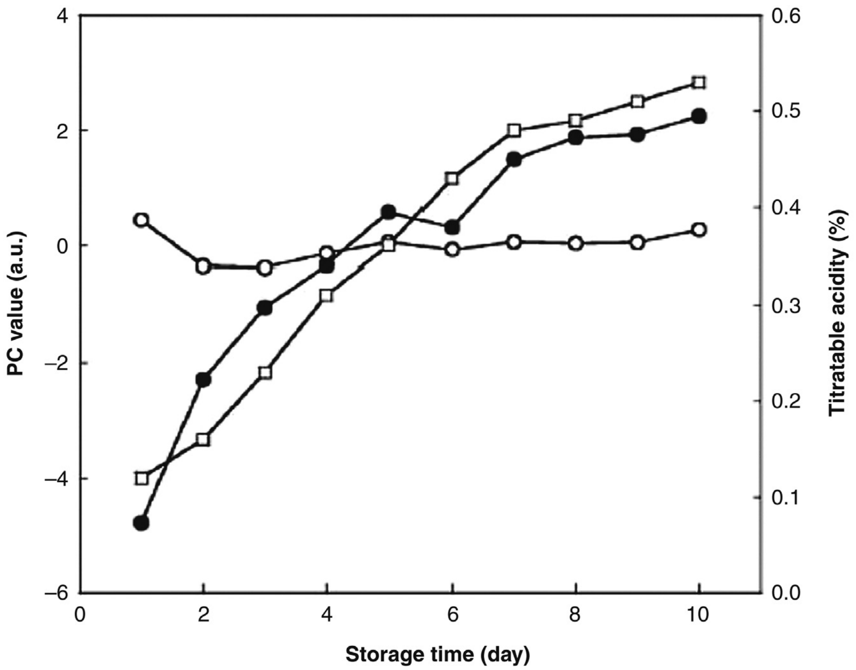

**FIGURE 22.5 Changes in the values of PCs and titratable acidity of the analytical samples prepared from baek-kimchi during the storage for 10 days at 4°C in the case of the cation-selective polymer membranes.** Symbols: (●) PC1; (○) PC2; (□) titratable acidity. *(Reprinted from Kim et al., 2005, with permission from Elsevier.)*

inexpensive instrumentation. At the same time, the use of systems instead of discrete sensors allows dealing successfully with such traditional problems of the discrete sensors as insufficient selectivity in the multicomponent media.

Electronic tongues offer the possibility to performing recognition and classification, and quantification of several components simultaneously in the fermentation broths. Thus, the electronic tongues can be employed for the process follow-up: to measure quantitatively content of some components and, at the same time, to assess the state of the process and its correspondence to the normal operation conditions. Several successful applications of the electronic tongues to food fermentation monitoring were reported in the literature during the last two decades. However, these applications are still confined to the laboratory. Transfer to this instrument to the industry is hindered by practical issues arising with sensor use in the multicomponent media such as sensors' contamination and, consequently, drift of their characteristics as well as necessity of regular recalibration. Evidently, these important practical issues must be addressed to make electronic tongues widely applicable to the routine fermentation monitoring in industry.

## ACKNOWLEDGMENT

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## Chapter 23

# Phenolic Compounds Analyzed With an Electronic Tongue

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## 23.1 INTRODUCTION

Electronic tongues (E-tongues) are novel analytical systems based on multisensory devices that develop applications in the analysis of samples of complex nature, in the recognition of their characteristic properties, or in the equivalent estimation of human taste perception (Ciosek and Wroblewski, 2007; del Valle, 2010; Riul et al., 2010). E-tongues have as main advantages their ability to provide comprehensive information of a sample with reduced effort, within a few seconds, and simultaneously detecting a large spectrum of compounds, all in one step.

Besides the wide variety of applications dealing with those reported in different fields (Ciosek and Wroblewski, 2007; del Valle, 2010; Riul et al., 2010), there are two key factors that determine the information obtained by those; that is, the type of sensors forming the sensor array and the chemometric tool used to process the high dimensionality data which is generated. On the one hand, between the different families of chemosensors that may form the E-tongue, potentiometric, voltammetric, or impedimetric sensors are the main variants used (del Valle, 2010), although optical or piezoelectric sensors might also be used. Furthermore, one of the recent advances in the design of E-tongues has been the incorporation of biosensors into the sensor array in order to tackle new application fields or to improve the existing ones; this approach has been named BioE-tongue (Tønning et al., 2005). Besides, it is even possible to combine the responses obtained from different sensor families, a strategy known as data fusion (Klein, 2004). On the other hand, within the whole range of chemometric tools that can be used, we can classify those according to the extracted information and the learning machine tool used (Richards et al., 2002). Namely, in the former case we will distinguish between qualitative and quantitative analysis, depending on the type of information that the processing tool allows us to extract; whereas in the latter, a distinction is made between supervised and unsupervised methods, depending on whether they are trying to find hidden structures in unlabeled data or inferring a response model from *a priori* known training data. Therefore, methods are categorized according to the information provided to the learning tool during the processing of the data.

Up to now, one of the major fields of application of E-tongues (and electronic noses) has been food and beverage analysis (Ciosek and Wroblewski, 2007; Escuder-Gilabert and Peris, 2010; Riul et al., 2010). Most of the applications described include the identification and classification of beverages and food variants, or perceptions of food quality or variety, even as a means to provide an automated equivalent to a sensory panel of human experts (Deisingh et al., 2004; Escuder-Gilabert and Peris, 2010; Scampicchio et al., 2008).

In this direction, among the different types of samples analyzed, special attention has been attracted toward wine and its related products (Zeravik et al., 2009). From the analytical point of view, wine is a complex mixture of diverse substances which exhibit considerable influence on its taste and characteristics. Among their constituents, phenolic content is important given their antioxidant properties (acting as free radical scavengers and inhibitors of lipoprotein oxidation (Sánchez-Moreno et al., 1999)) and their simultaneous effect in wine organoleptic and sensorial properties (Ribéreau-Gayon et al., 2006).

E-tongues have been successfully applied to the analysis of wines and musts. On this respect, reported publications are aimed mainly to discrimination of samples of different varieties/origins (ie, classification tasks) (Gay et al., 2010; Gutiérrez et al., 2010a; Riul et al., 2004), to prediction of certain chemical properties (eg, total acidity, organic acids, ionic composition or phenolic compounds, between others) (Gay et al., 2010; Legin et al., 2003) or taste attributes in comparison to a sensory panel (Cetó et al., 2015). Besides, there are also some interesting works focused

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on the detection of inappropriate handling practices or adulteration processes (Gutiérrez et al., 2010b; Parra et al., 2006), the use of alternative aging methods (Gay et al., 2010), and the monitoring of alcoholic fermentation (Buratti et al., 2011).

Among the different applications reported, this chapter will specifically focus on E-tongues usage for the analysis of phenolic compounds in wine. This beverage contains a wide variety of phenol-related molecules, which may depend on the type of grape, harvest, elaboration, and aging. On that account, in the next sections we will first provide an overview of the more significant contributions found in the literature, then we will better illustrate E-tongues capabilities with selected applications carried out in our laboratories devoted to the analysis of phenolic compounds in wine.

## 23.2 EXAMPLES ON THE LITERATURE

Many variants of E-tongues have already been used for the detection of phenolic compounds, including the use of sensors of potentiometric (Rudnitskaya et al., 2010a), voltammetric (Gutés et al., 2005) and impedimetric nature (Olivati et al., 2009). Moreover, E-tongues have not only been applied to wine, but also to beer (Polshin et al., 2010), oil (Apetrei et al., 2010), tea (Papieva et al., 2011), or different fruit juices or cocoa (Huang et al., 2014), as well as other beverages. However, as previously stated, in this chapter we will focus in their applications toward wine samples.

On that account and focusing on the key parameters already discussed (namely, the type of sensors and the chemometric tool), different types of sensors have been employed to tackle phenolic compounds determination. Therefore, the sensor array does not represent a limitation by itself, although best results have been achieved through the use of voltammetric sensors; more specifically, with voltammetric biosensors, an approach known as BioE-tongue (Cetó et al., 2012a). However, the use of voltammetric (bio)—sensors is one of the most complex cases given the more elaborated nature of the sensors used and the high dimensionality of the considered signals which hinder its treatment, usually requiring the usage of a preprocessing stage (Cetó et al., 2013). This preprocessing, normally a feature extraction step, is needed to reduce the complexity of the input signal while preserving the relevant information; this in addition allows avoiding redundancy in input data, reducing training time, and obtaining a model with better modeling accuracy, higher robustness plus better generalization ability and easier operation and understanding (Cetó et al., 2013).

Moreover, we can also distinguish between qualitative and quantitative approaches, that is, the ones that address the classification or discrimination of samples based on its phenolic content, and the ones that focus on the quantitative determination of phenolic compounds. In the latter, we might also distinguish between three different scenarios, namely, the quantification of total phenolic content in the form of a global index, the quantification of total phenolic subgroups/families content (eg, tannins and/or anthocyanins) and the simultaneous quantification of selected individual phenolic compounds.

In this sense, most of the reported E-tongue works focus on the determination of total phenolic content (the first scenario), concretely toward the prediction of the two global indices most commonly employed: the Folin–Ciocalteu index (FC) and the UV polyphenol index ($I_{280}$) (Waterhouse, 2001). The first one is a colorimetric assay that measures the amount of phenolics (usually expressed as equivalents of gallic acid) needed to inhibit the oxidation of the Folin–Ciocalteu reagent (a mixture of phosphomolybdate and phosphotungstate, which are reduced to the respective oxides). The second index is a direct measurement of the absorbance of the sample at 280 nm, which is related to phenolic concentration since all phenolic compounds absorb UV light, and also present some absorbance at 280 nm. As said, many examples reporting the correlation with one of those indexes (or both) can be found in the literature (Cetó et al., 2012c; Verrelli et al., 2007). Although in many cases those works are not limited to total phenolics index quantification, but to more complex scenarios like the works described later, and just the quantification of those indexes are a first approach to prove the capabilities of the system. Among the analytical methods, electroanalytical techniques are of special interest, given the antioxidant activity of phenolics is directly related to their electrochemical properties.

As for the first type of application, dealing with the classification of samples, we can find some reports that tackle the discrimination of individual phenolic compounds (Gay Martín et al., 2012), but also some that relate those to the discrimination of different grape varieties (Medina-Plaza et al., 2014) or to the bitter (Rudnitskaya et al., 2010a) and astringent taste (Puech et al., 2007), in all cases departing from phenolics content.

Moving forward to the second scenario, and in between the total and the individual quantification of specific phenolic compounds, there are some works focusing in the correlation of E-tongue measurements with the quantification of some specific phenolic subgroups. That is, phenolic compounds in wine include a large group of chemical species: phenolic acids, stilbenoids, flavonols, dihydroflavonols, anthocyanins (the red pigments in the grapes), flavanol monomers (catechins), and flavanol polymers (proanthocyanidins), which can be broadly separated into two main categories: flavonoids and nonflavonoids (Ribéreau-Gayon et al., 2006). Within the more important ones, flavonoids include the anthocyanins and tannins (relevant in the structure, body and taste of wine);

whereas nonflavonoids include the stilbenoids such as resveratrol, and phenolic acids such as benzoic, caffeic, and cinnamic acids. Hence, applications can be developed focusing on the quantification of any of those groups of species. In this matter, some interesting works are the ones reported by Professor Rodríguez-Méndez's team, which dealt with the correlation with catechins, anthocyanins, or tannins content (Gay et al., 2010; Rodriguez-Mendez et al., 2014), or also another work in which qualitative discrimination between the tannins subfamily was achieved (Puech et al., 2007).

Lastly, in what can be considered the more difficult and complex of the discussed approaches, there is the individual quantification of different phenolic compounds, which represents an application comparable to much more complex analytical techniques such as high-performance liquid chromatography (HPLC). Although being the less explored field, and still requiring of some advances to fulfill industry requirements, there are very promising works that deserve special attention. In this direction, correlation of E-tongues measurements with individual phenolic acids such as gallic and caffeic acids has been reported, either from wine (Kirsanov et al., 2012) or from its cork (Rudnitskaya et al., 2006), but also to a total of 6 different compounds from a list of 15 different ones (Rudnitskaya et al., 2010b).

At this point it would be interesting to point out that E-tongues might be considered an attractive alternative to conventional methods, with many advantages over those such as low-cost, portability, or fast-response, between other interesting characteristics, which might even allow to perform on-field measurements, even at the vineyard. E-tongues can therefore add an aspect of versatility because they can carry out both the determination of total phenolic content as well as the individual speciation of either specific groups of those or individual compounds. This represents an advantage by itself because the same device might allow both tasks without the requirement of heavy laboratory equipment, while with conventional methods each of those might be achieved separately. That is, on one hand, we have methods such as Folin–Ciocalteu index, which yields a total phenol content value, but does not discriminate between individual phenols; while on the other hand, we have methods such as HPLC or GC (gas chromatography), which allow the speciation of phenols, but do not measure the total content. Even if we are trying to calculate the total content from the sum of the individual compounds measured by chromatographic methods, the latter appears to be inferior. This could be partially explained by the lack of data on phenolic compounds in the literature, and also in food composition tables, but also by the difficulty to measure certain phenolics by chromatographic methods and the reactivity of the Folin–Ciocalteu assay with nonphenolic reducing compounds, prone to its overestimation.

## 23.3 VOLTAMMETRIC BIOE-TONGUE

In order to better illustrate the capabilities of E-tongues in this field, this chapter will now present some applications carried out in our laboratories devoted to the analysis of phenolic compounds in wine (Fig. 23.1); steeply going from the quantification of total phenolic content to the individual identification of certain individual phenolics. Therefore, demonstrating that, with an appropriate set of samples and training of the system, an E-tongue might be able to provide both types of information. Additionally, what is even more interesting, not requiring any extra samples measurement step, as recorded responses might just be used as input into the different built models.

More specifically, in the first example, the quantification of total phenolic content in wine samples employing a BioE-tongue will be presented, that is, with a sensor array formed by voltammetric enzyme-modified biosensors; in a next step, individual discrimination of phenolic compounds will be tackled, thanks to the BioE-tongue's superior performance. In this account, qualitative discrimination will be first evaluated, then, in a more complex approach, the quantitative determination of individual phenolic compounds in ternary mixtures will also be described.

### 23.3.1 Sensor Array

The voltammetric (bio)sensors used were bulk-modified graphite-epoxy composites, which is the usual configuration of our laboratories (Céspedes et al., 1996). In this way, an array of four (bio)sensors was prepared comprising one blank electrode plus three different bulk-modified ones, using bare graphite C and adding as modifiers enzymes such as tyrosinase and laccase, and copper nanoparticles.

In our case, tyrosinase and laccase were chosen as the enzymes employed to detect the phenolic compounds. These enzymes belong to the class of copper-containing oxidases, which catalyze the reduction of molecular oxygen by different phenolic electron donors. In those reactions, the oxygen is reduced directly to water without the intermediate formation of hydrogen peroxide. Similarly, copper nanoparticles were chosen given that both tyrosinase and laccase are copper-containing enzymes. Then, it was thought that some catalytic effect could be derived from those, a hypothesis that was confirmed when observing the sensor's response (Fig. 23.2).

### 23.3.2 Measurements

Electrochemical experiments were carried out at room temperature under quiescent conditions in a multichannel configuration, without performing any sample pretreatment or physical regeneration of the working electrodes. In this sense, to prevent any accumulative effect

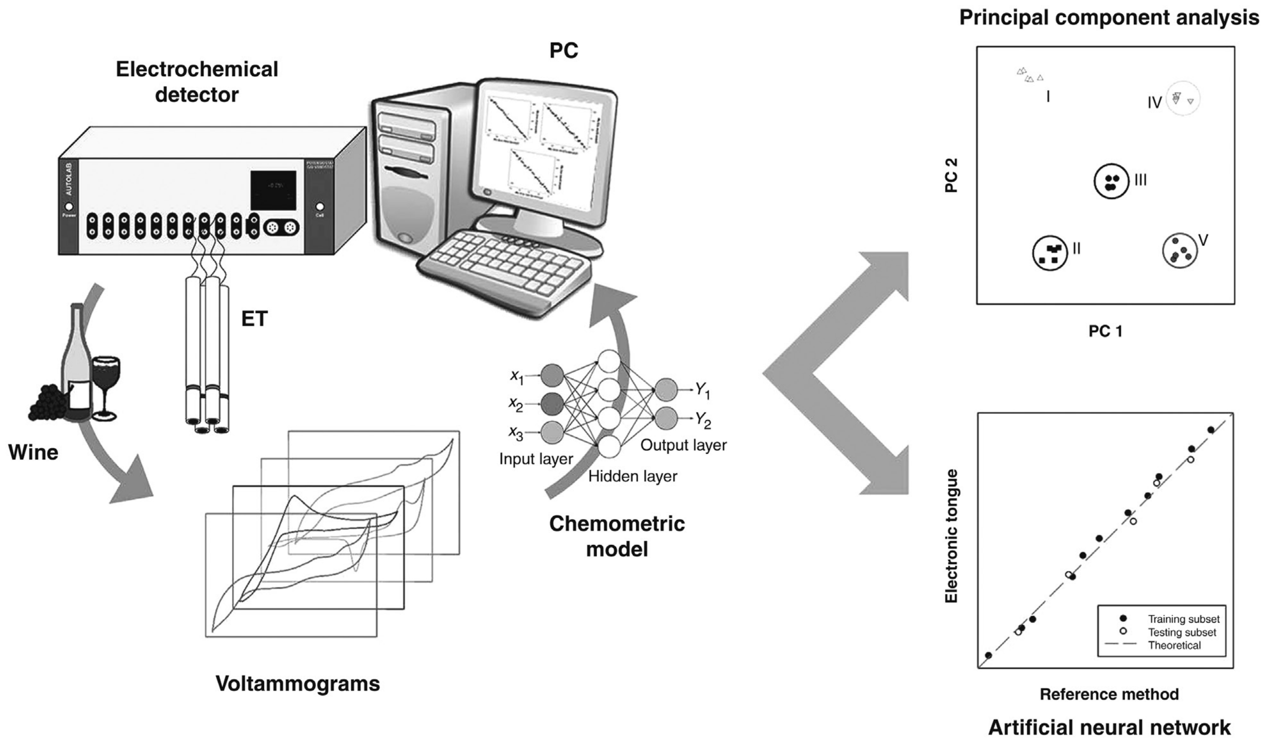

**FIGURE 23.1** **Processing scheme of the electronic tongue approach.** Samples are measured employing the sensor array, and obtained responses are then preprocessed and entered to the chemometric model, which allows the prediction of the desired outputs, either qualitative or quantitative parameters.

of impurities on the working electrode surfaces, an electrochemical cleaning stage was done between each measurement by applying a higher conditioning potential in a cell containing distilled water (Cetó et al., 2011). This step minimized electrode fouling and recovered the original signal (baseline).

### 23.3.3 Chemometric Analysis

Chemometric analysis was done by specific routines written by the authors in MATLAB, using its Neural Network Toolbox. Particularly, principal component analysis (PCA) was used for qualitative analysis of the results, while quantitative analysis was achieved by means of artificial neural networks (ANNs).

Furthermore, due to the large dimensionality of the generated data, a preprocessing stage for data reduction, employing fast Fourier transform (FFT) was also performed (Cetó et al., 2013). The main objective of this step is to decrease the dimensionality and complexity of the electrochemical signatures while preserving the relevant information, what in addition allows to gain advantages in training time, to avoid redundancy in input data and to obtain a model with better generalization ability (Cetó et al., 2013).

### 23.3.4 Voltammetric Responses

An extract of the typical response profile of the employed BioE-tongue array is presented in Fig. 23.2. As expected from an array aimed to phenolic compounds detection, a clear voltammetric response toward them (with currents increasing as their concentration increases) can be seen (Fig. 23.2a, b), besides still showing a differentiated response toward different individual compounds (Fig. 23.2c, d). Moreover, also demonstrating the improvement derived from the use of biosensors, as, for example, the higher signal attained with those, especially in the region close to 0 V.

Therefore, the proposed BioE-tongue generates enough rich data that can be a useful departure point for the multivariate calibration model; that is, obtaining differentiated signals for the different electrodes, and with those being related to the phenomena under study. However, due to the complexity of the departure data, a preprocessing step for data compression will be required prior to its modeling as previously described.

### 23.3.5 Total Phenolic Content

As for the first attempt, the quantification of total phenolic content in wine was undertaken. The reason was to begin

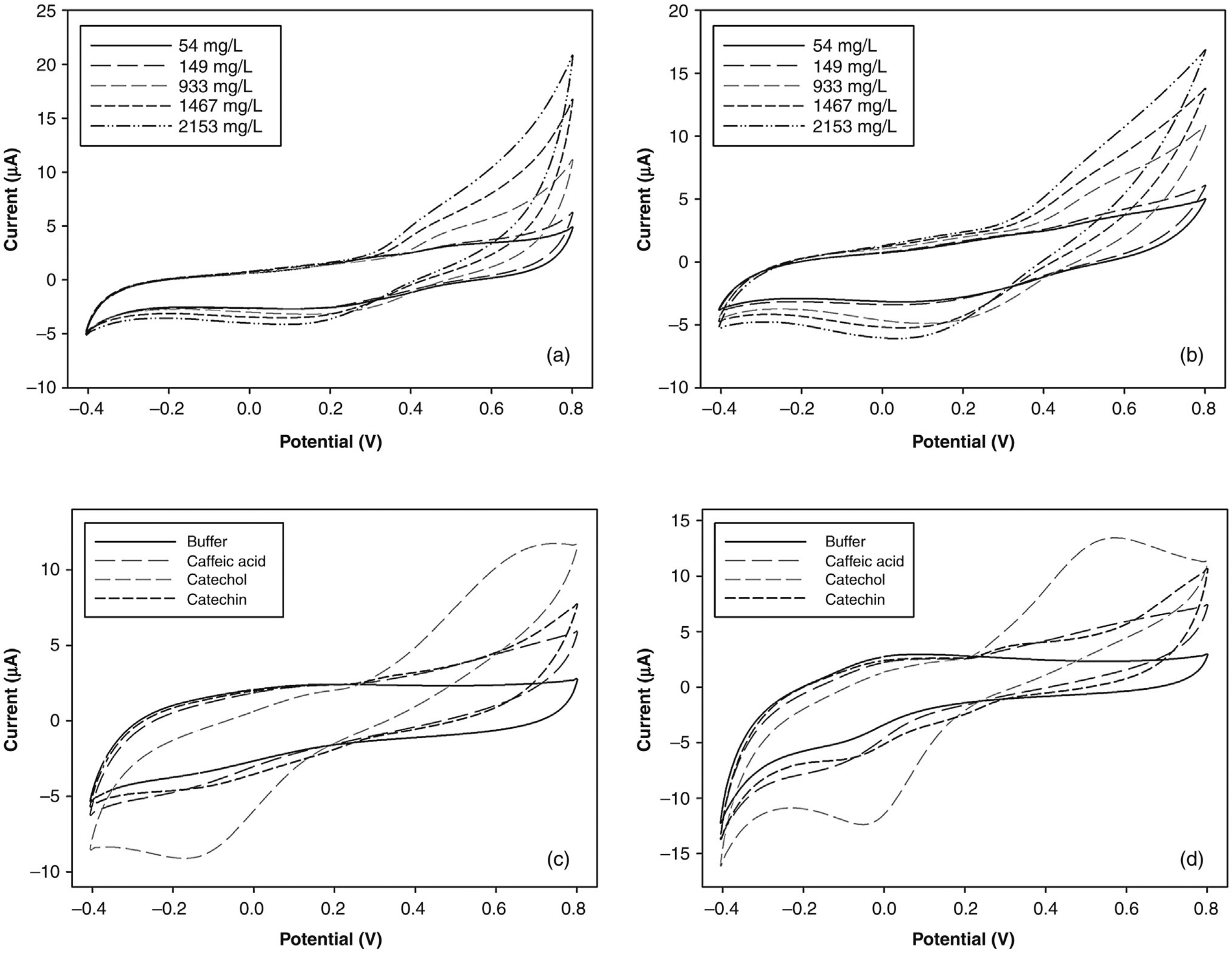

**FIGURE 23.2** (a, b) Example of the responses obtained with the BioE-tongue for certain arbitrary wine samples with graphite-epoxy sensor and tyrosinase biosensor, respectively; Folin–Ciocalteu indexes are expressed in equivalents of gallic acid; (c, d) voltammetric responses obtained with the BioE-tongue for certain phenolic compounds stock solutions with tyrosinase biosensor and copper nanoparticle modified sensor, respectively. *(Reprinted from Cetó et al., 2012a, with permission from Elsevier.)*

with what might represent the easiest of the discussed scenarios, given the lower selectivity requirements; afterwards, the complexity of the different studies would be increased gradually up to achieving the individual quantification of specific phenolic compounds.

In this direction, the set of samples considered for this first study was formed by a total of 29 wine samples of different varieties and from different Spanish regions in order to obtain a data set with sufficiently differentiated total phenolic content, as well as from different harvests and grape varieties. Additionally, and for comparison purposes, its total phenolic content was assessed spectrophotometrically with the reference method (the more common one), namely Folin–Ciocalteu index (Waterhouse, 2001). Hence, the goal was the correlation between E-tongue measurements and that index.

The set of samples was then measured with the voltammetric BioE-tongue and obtained responses were preprocessed employing FFT, using the obtained coefficients as inputs to the ANN model in order to achieve the prediction of the total phenolic indexes in wines. First, a significant effort was needed to optimize the operation details that determine the ANN configuration; this is a trial-and-error process, where several parameters (training algorithms, number of hidden layers, transfer functions, etc.) are fine-tuned in order to find the best configuration that optimizes the performance of the model. This optimization procedure is usually carried out by employing a subset of the samples considered, keeping some of them aside to evaluate its actual performance. Therefore, this step is important because this division can lead to biased results, depending on the specific subdivision of the data. To this aim, accuracy of the generated model was evaluated by employing the repeated random subsampling validation method (Molinaro et al., 2005), training with 80% of the data (23 samples) and testing with the remaining 20% (6 samples). In this way, train/test data subdivision was repeated randomly 29 times (as many times as samples, similar to *k*-fold method) in order to ensure that the model's accuracy is good enough and BioE-tongue performance does not depend on the specific subsets used. Then, once all responses from all the constructed models were obtained, predicted values by each model were grouped, depending if they were used in the training process or in the testing subset (again, similar to *k*-fold method). Finally, average values for each sample were calculated, allowing us to calculate model uncertainties and to obtain unbiased average data (Riu and Bro, 2003).

Next, comparison graphs of predicted versus expected indexes were built, both for training and testing subsets, to check the model prediction ability (Fig. 23.3). As can be observed, a good trend was obtained for the Folin–Ciocalteu index, with regression lines almost indistinguishable from the theoretical ones and small confidence intervals for each of the predictions. Regression parameters were also calculated, and as expected from the comparison graphs, fitted comparison lines were close to the ideal values, with intercepts close to 0 and slopes and correlation coefficients close to 1, meaning there were no significant differences between expected and calculated index values.

Therefore, these results demonstrated how E-tongues can represent an alternative to standard methods that provide polyphenol global indexes, with advantages over those such as a reduction in analysis time, avoiding sample pretreatment (proper dilution factor), and the use of reagents (Folin–Ciocalteu reagent and sodium carbonate). It also allows the simultaneous determination of the global index, as well as other indexes such as the $I_{280}$, the trolox equivalent antioxidant capacity (TEAC), or tannins and anthocyanins phenolic subfamilies, with proper training of the system (Gay et al., 2010).

## 23.3.6 Individual Discrimination of Phenolic Compounds

Nowadays there is an increasing demand for highly sensitive, selective, and fast-response analytical methods that, apart from being able to provide a global index of the total phenolic content, are also able to carry out their individual determination (Ignat et al., 2011). In this respect, despite the huge efforts being carried out in this field, the separation and quantification of individual phenolic compounds remains difficult; particularly the simultaneous determination of different chemical subgroups.

Fig. 23.4 shows the majority of phenolic compounds present in wine. Phenolics in wine originate from benzoic acid (gallic acid), cinammic acid (caffeic and ferulic acids), stilbenes, flavonols (quercetin) and flavanols (catechin), as well as some other condensed forms (Ribéreau-Gayon et al., 2006). Given that wine is a significant source of polyphenols in the diet, the characterization

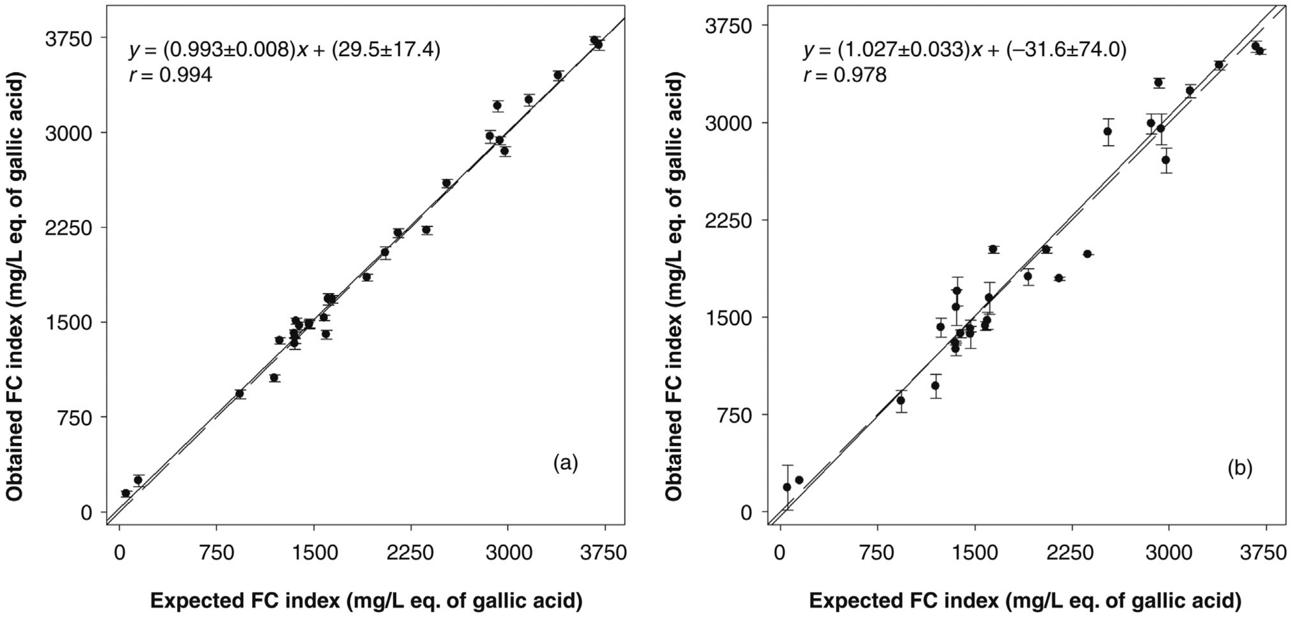

**FIGURE 23.3 Modeling ability of the optimized FFT–ANN.** Sets adjustments of expected versus obtained Folin–Ciocalteu indexes for (a) training and (b) testing subsets, respectively. The *dashed line* corresponds to the theoretical diagonal line. Results provided correspond to the average of the values obtained for each sample after 29 repeated calculations, done with random division of samples for train/test subsets each time. Uncertainties calculated at the 95% confidence level. *(Reprinted from Cetó et al., 2012a, with permission from Elsevier.)*

and determination of phenolic compounds in wine is an important concern in this field. Phenolic compounds in wine can reach values of 4 g/L in the heaviest red wines (Frankel et al., 1995), and have been claimed, especially thanks to their antioxidant properties, to have beneficial properties of protecting low-density lipoprotein (LDL) levels more effectively than other antioxidants, such as alfa-tocopherol, and then being more effective protective agents against arteriosclerosis (Sánchez-Moreno et al., 1999). Other phenolic compounds existing in wine, for example, quercetin or resveratrol, have been claimed to display anticancer effects (Angst et al., 2013). Phenolic compounds are also important for their organoleptic properties, giving astringency, odor, and savor to wine (Ribéreau-Gayon et al., 2006); they are similarly important in exactly this role for many other vegetal food products (like olive oil, tea, coffee, or other fruit products).

Thus, the beverage industry may find important advantages if fast, easy methods are available for polyphenol characterization. In this direction, the next examples will attempt the individual discrimination of different phenolics; first, through the qualitative analysis of different wine-spiked samples with different individual compounds as a first approach to evaluate the capabilities of the BioE-tongue in such task, and second toward the simultaneous determination of ternary mixtures of majority species.

### 23.3.6.1 Qualitative Discrimination

Prior to proceeding to the quantification of the phenolics mixtures, we need to confirm that differentiated responses have been obtained for the different compounds and that the wine matrix does not represent a problem by itself, hence generating enough rich data that can be a useful departure point for the multivariate calibration model.

To confirm this differentiated behavior and to assess the ability of the BioE-tongue to discriminate between them, a first qualitative attempt was carried out by analyzing some spiked wine samples with a small quantity (ca. 36 mg/L) of typical phenolics normally present in the samples; concretely, considered compounds were: gallic acid, (±)-catechin, *p*-coumaric acid, caffeic acid, catechol, phenol, *m*-cresol, ferulic acid, chlorogenic acid, and quercetin (Fig. 23.4). Besides, raw wine (nonspiked) and replicated samples were considered and analyzed randomly to be sure that (dis)similarities observed in the PCA plot are not a consequence of the order in which samples were analyzed.

Hence, a total set of 55 samples distributed in 11 classes were analyzed with the BioE-tongue array, and obtained

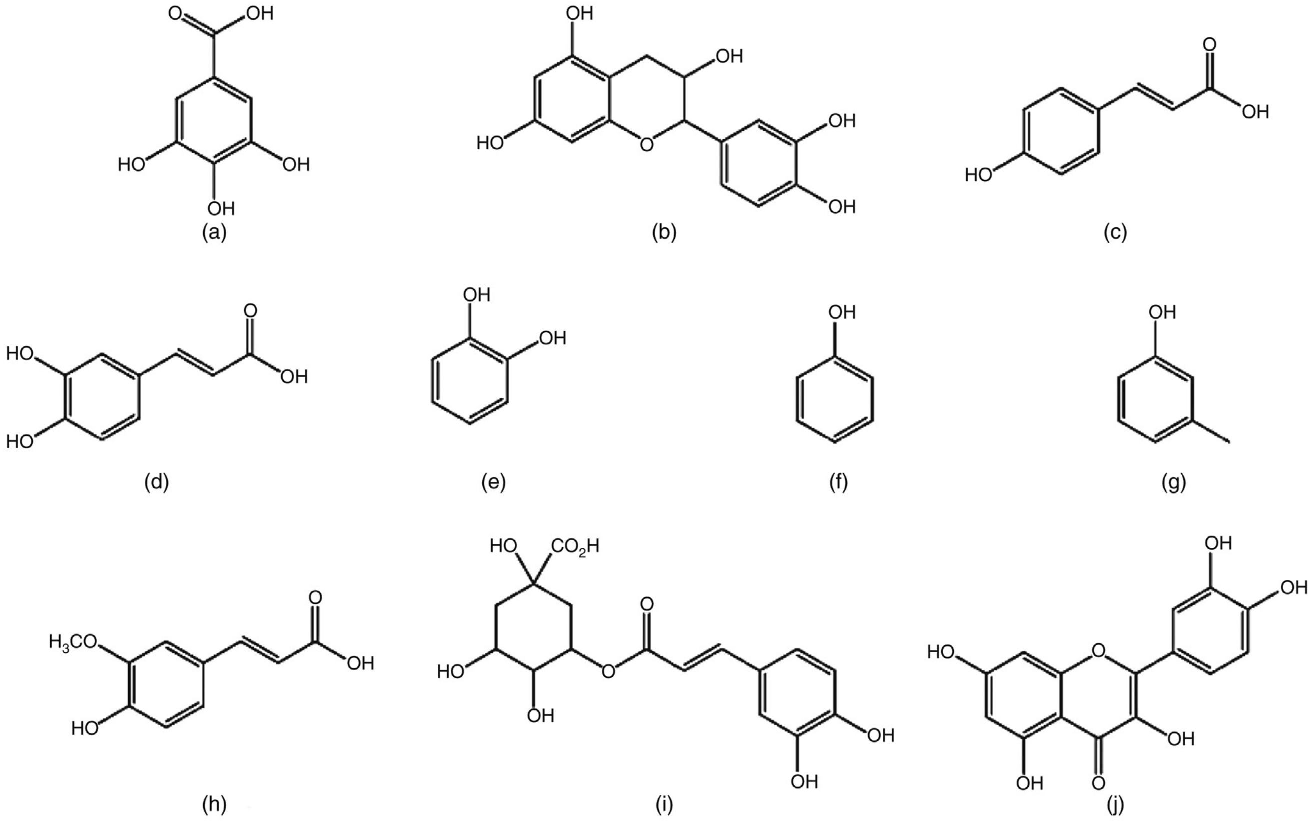

**FIGURE 23.4 Structure of some phenolic compounds present in wine.** (a) Gallic acid, (b) catechin, (c) *p*-coumaric acid, (d) caffeic acid, (e) catechol, (f) phenol, (g) *m*-cresol, (h) ferulic acid, (i) chlorogenic acid, and (j) quercetin.

responses were processed by means of FFT for its compression and PCA to obtain a better visualization of the data variability and check if the samples group together according to the spiked phenolic compound.

The PCA score plot is shown in Fig. 23.5, where the obtained patterns evidence that samples are grouped according to the spiked phenolic compound, with clusters that clearly separate all the samples. The accumulated explained variance with the three first PCs was ca. 99.2%, a huge value that guarantees that almost all the variance contained in the original data is now represented with these new three variables.

Analyzing the plot more deeply it can be seen how wine appears in the central position, from which spiked samples group around. On the right side (with negative values for PC1), we find the major phenolics found in wine (clusters I–IV); while on the other side (with positive values for PC1), and closer to each other, we find the remaining studied compounds, which are usually less abundant. Besides, it should also be taken into account that the position of the clusters will also be highly related to the different affinity of the enzymes toward each of the compounds.

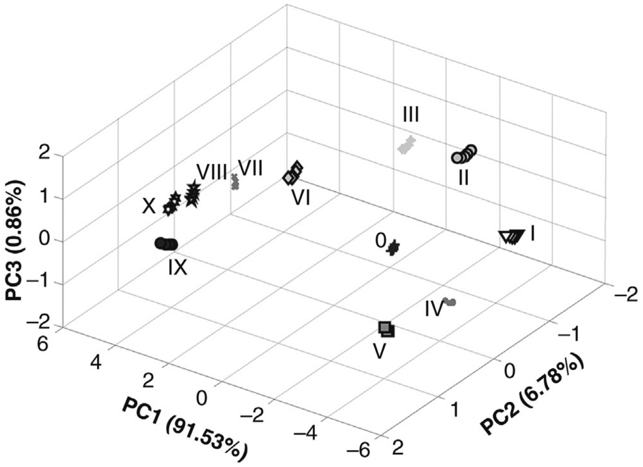

**FIGURE 23.5** Score plot of the first three components obtained after PCA analysis of the spiked wine samples corresponding to: (0) wine, (I) gallic acid, (II) (±)-catechin, (III) *p*-coumaric acid, (IV) caffeic acid, (V) catechol, (VI) phenol, (VII) *m*-cresol, (VIII) ferulic acid, (IX) chlorogenic acid, and (X) quercetin.

### *23.3.6.2 Quantitative Mixtures Resolution*

Given the trend observed in the previous study case, the next step was to move from qualitative to quantitative discrimination; that is, to achieve the individual determination of each of the phenolics in their mixtures. Concretely, in this case, toward the simultaneous determination of caffeic acid, catechol, and catechin mixtures because these are three of the major phenolic compounds found in wine (Ribéreau-Gayon et al., 2006).

To this aim, BioE-tongue performance was first evaluated for a set of 27 manually prepared standards based on a $3^3$ cubic design used to establish the response model (training subset) (Cetó et al., 2012b), plus 10 additional samples distributed randomly among it and used to evaluate its performance (testing subset), that is, a total set of 37 samples. Using this set of samples, the ANN model was adjusted, and comparison graphs of predicted versus expected concentrations for each of the three phenols were built to evaluate its modeling and prediction capabilities (Fig. 23.6). Then, regression parameters were also calculated, and as expected from the comparison graphs, a good linear trend was attained for all the cases, with results obtained for both subsets close to the ideal values, that is, with slopes and correlation coefficients close to 1 and intercepts close to 0, with all of them included in the confidence intervals.

Once that the BioE-tongue performance for the resolution of phenolics mixtures was proven using standards, and in order to test its applicability to real samples, some wine samples were again spiked with variable amounts of the

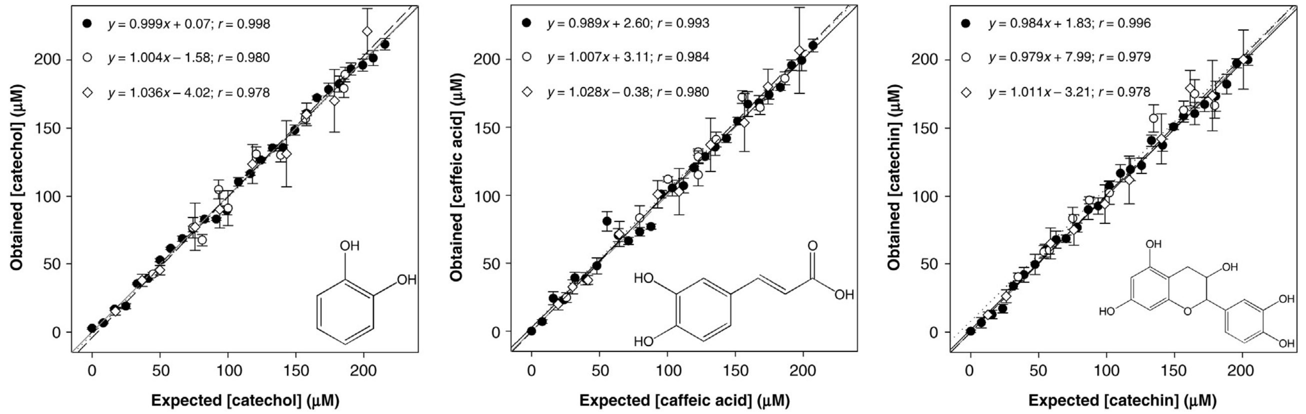

**FIGURE 23.6** Modeling ability of the optimized FFT-ANN for (●, *solid line*) the training subset, (○, *dotted line*) the test subset, and (◊, *short-dashed line*) the wine samples. Set adjustments of obtained versus expected concentrations for caffeic acid, catechol, and (±)-catechin (left to right). The *dashed line* corresponds to theoretical diagonal line, and error bars correspond to five different retrainings with random reinitialization of weights. *(Adapted from Cetó et al., 2012b, with permission from the Royal Society of Chemistry.)*

three phenolics considered. Employing the same ANN architecture with the leave-one-out cross-validation method, forced by the reduced number of samples and to ensure that each sample in the set is used in the validation step, the system was retrained. Then, prediction capability of the ANN model for wine samples was evaluated. As before, comparison graphs of predicted versus expected concentrations for the three determined phenols were built (Fig. 23.6); again, obtaining a good trend for the three phenolics, with regression lines almost indistinguishable from the theoretical ones.

## 23.4 CONCLUSIONS

Electronic tongues have been proven to be a promising tool for the analysis of phenolic compounds, either for the determination of its total content or for the speciation of individual compounds or groups of compounds, thus, providing a fast-response and low-cost method for blind samples characterization, and really suitable as a screening method for quality control.

Besides representing an attractive alternative to conventional methods, with many advantages over those such as its low-cost, portability, or fast-responses, between others. One of the main benefits of E-tongues lies in their ability to carry out both the determination of total phenolic content as well as the individual speciation of either specific groups of those or individual compounds. Therefore, this represents an advantage because the same device might allow both tasks without requiring heavy laboratory equipment, whereas with conventional methods each task might be achieved separately.

In this fashion, E-tongues represent a response to the demand of new analytical methods with high sensitivity, good selectivity, and fast response needed to meet new challenges in food analysis. In addition, E-tongues have become a technique extremely simple in operation, able to easily overcome limitations found with classical approaches in sensor research, allowing its usage without any sample pretreatment and thus representing an interesting alternative to more sophisticated methods as with the chromatographies.

## ACKNOWLEDGMENTS

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## Chapter 24

# Electronic Tongue for the Estimation of Important Quality Compounds in Finished Tea

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## 24.1 INTRODUCTION

The exotic aroma and refreshing taste of tea makes it one of the most consumed beverages in the world. The taste and aroma quality of tea is the result of subtle and multidimensional contribution of innumerable chemical constituents present in the tea. While the volatile compounds define the quality of aroma, the nonvolatile compounds determine the taste and cup character of tea liquor. It is known that nearly 700 volatile compounds constitute the tea aroma and large numbers of nonvolatile compounds are responsible for taste formation in tea liquor (Wood and Roberts, 1964).

Regarding the taste of tea liquor, the most important group of compounds present in green tea leaves are polyphenols. These are bioactive compounds with diverse biological properties as they act as antifeedants, antioxidants, phytoalexins, attractants for pollinators, contributors to plant pigmentation, and protective agents against UV light. These compounds possess one or more aromatic rings bearing single or multiple hydroxyl groups and possess wide structural variations ranging from that of a simple phenolic molecule to those of complex high molecular mass polymers (Balasundram et al., 2006). Polyphenols are divided into several classes according to the number of phenol rings that they contain and the structural elements that bind these rings to one another. The main groups of polyphenols are: flavonoids, phenolic acids, tannins, stilbenes, and lignans. Flavonoids are the most widely found phytochemical with strong antioxidant and antistress properties. They are found to be beneficial for human health. The flavonoids may be structurally classified into flavonols, flavones, flavanones, flavanols (or catechins), isoflavones, flavanonols, and anthocyanidins. The polyphenols constitute 25–30% of a fresh tea shoot and are known to produce a pungent astringent taste in tea liquor. The most important polyphenols present in tea leaves leading to the formation of taste compounds in tea are flavan-3-ols (Obanda et al., 2001; Owuor et al., 2006), namely,

1. catechin (C)
2. epicatechin (EC)
3. epigallocatechin (EGC)
4. epicatechin-3-gallate (ECG)
5. epigallocatechin-3-gallate (EGCG)

These compounds differ in their chemical structure, electrochemical behavior, and astringency contribution in tea liquor. They can be categorized according to the hydroxyl groups on the B-ring (C, EC, and ECG are dihydroxilated, while EGC and EGCG have trihydroxilated B-ring) or as gallated or nongallated catechins (C, EC, and EGC are nongallated, while ECG and EGCG are gallated). However, the simple, nongallated tea catechins EC, EGC, and C are not as astringent as the gallated catechins ECG and EGCG.

The objective of tea processing steps is to initiate essential chemical reactions at every stage of processing so that the polyphenols in green tea leaves are maximally transformed to desirable taste inducing compounds. The moisture induced stress on tea leaves during withering activates principal oxidative enzymes like the polyphenol oxidase (PPO) and peroxidase (PO). The conditions for the most important chemical reactions are set up during this stage. The catechins are localized in the vacuoles of plant cells, while the oxidative enzymes are associated with the chloroplasts. During the leaf maceration stage, the catechins come in contact with the oxidizing enzymes. The oxidative transformations of catechins start immediately from this step and gain momentum during the fermentation stage. The primary oxidation pathway involves the transformation of the phenolic substances to quinones. The quinones are again converted (condensation and polymerization) into a

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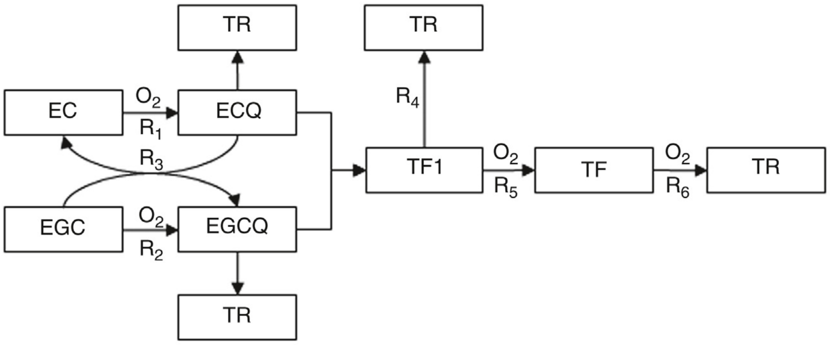

**FIGURE 24.1 Biochemical reactions during black tea fermentation process.** *EGC*, Epigalocatechin; *EC*, epicatechin; *ECQ*, epigalocatechin-quinones; *EGCQ*, epicatechin gallate-quinones; *TF*, theaflavins; *TF1*, intermediate theaflavins; *TR*, thearubigins; and *R1–R6*, reaction serial numbers.

group of compounds called the theaflavins (TF) and thearubigins (TR). The low molecular weight variants (fractions) of TF and TR are initially produced and are converted into high molecular weight variants as the fermentation process is prolonged. TR is also converted from TF at the later part of fermentation. TF and TR are the characteristic pigments with dominant taste contributory properties and are produced during processing. In other words, these compounds are responsible for imparting color and taste to tea. The schematic of reaction pathways for the formation of TF and TR from catechins during the oxidation (fermentation process) of tea manufacture is presented in Fig. 24.1.

TFs are low molecular weight compounds and golden yellow in color, while TRs are high molecular weight compounds and orange brown in color. These pigments are responsible for taste, strength, brightness, and color of black tea. It is known that TF and TR are the most important chemical compounds in tea liquor since the taste attributes like "briskness" and "strength" are mainly contributed by these two classes of compounds (Roberts, 1962). A significant relationship has been established between TF content and the price of tea (Roberts, 1958, 1962; Hilton and Ellis, 1972).

The quality of crush-tear-curl (CTC) type of tea is determined by the taste and color of its infusion. In this light, the presence of TF and TR directly influences the quality of CTC tea.

The presence of TF contributes to astringency and brightness of tea liquor, whereas the presence of TR is known to increase mostly the ashy taste with a slight improvement in astringency and reduction in brightness. TF constitute about 0.5–2% of dry weight depending on the processing parameters of tea, while TR constitute about 6–18% of dry weight. TF imparts briskness and brightness, while TR contributes to the mouth feel (thickness) and color of the tea (Biswas et al., 1973; Obanda et al., 2004). It may be noted that TR has an ashy taste and is only slightly astringent, while TF is astringent enough to affect the overall astringency of liquor and contribute to differences among quality of various clones. In other words, the taste threshold of TF is much lower than that of TR and polyphenols (Scharbert et al., 2004b). Spectrophotometric and human sensory panel studies suggest that TF content correlates positively with liquor brightness. The TR content, however, is found to relate negatively with liquor brightness (Roberts and Smith, 1963; Ngure et al., 2009). Although the concentrations of other chemicals like caffeine and amino acids also contribute to the quality of finished tea, the concentrations of TF and TR have much greater influence on the desirable qualities such as brightness, briskness, depth of color, strength, mouth feel, and overall quality of finished tea (Roberts, 1958, 1962; Biswas et al., 1971; Ramaswamy, 1962; Hazarika et al., 2002). The optimum taste perception of tea liquor is the result of delicate and subtle contribution of the previously mentioned biochemical components. As an example, high concentrations of polyphenols or TR deteriorate the taste of tea liquor. The TFs are the most important compounds that directly affect the taste of tea liquor in a positive manner.

It may thus be understood that the tea biochemicals like TF and TR have a very distinct effect on the palate of tea liquor and the estimation of their concentrations will give a consistent idea about the quality of CTC tea. It is therefore imperative to explore rapid quantification of those compounds using an electronic tongue (e-tongue), considering the fact that it has been previously used to analyze the tea samples.

## 24.2 ELECTRONIC TONGUE FOR TEA: THE CURRENT STATE OF ART

An electronic tongue is a biologically inspired sensory-electrical system in which the responses from electrodes with overlapping selectivities are recorded and interpreted using intelligent multivariate statistical models. The sample analytes from natural sources are typically characterized by a complex chemical matrix of innumerable compounds. In general, a large number of electrochemical sensors are available for specific detection of various chemicals. However, the feasibility of using such specific electrodes for each type of compound is severely limited by the sheer number of chemicals that constitute the analytes from natural sources (eg, tea). Under such situations, the electrodes with overlapping selectivity yield information about most of the compounds but with different levels of sensitivities. The multivariate statistical methods are employed to extract the desired quantity of interest from the electrical signatures obtained from the electrodes.

Until now, various types of e-tongues had been proposed for tea applications, with the operating principles encompassing potentiometry, voltammetry, amperometry, impedometry, and fluorometry. Initially, Ivarsson et al. (2001) proposed a voltammetric e-tongue made of three noble metal wire electrodes for the discrimination of various food samples, including nine different types of tea samples. Ivarsson and coworkers investigated different types of control waveforms for probing the tea extracts used as an electrolyte and resorted to the multivariate statistical method of principal component analysis (PCA) for illustrating that the response of e-tongue was different for each type of tea sample. Lvova et al. (2003) then used a potentiometric e-tongue system consisting of a solid-state disposable polymeric membrane based on carbon paste electrodes for classification of three different varieties of Korean green teas. The estimation of major tea compounds such as caffeine, tannic acid, sugars, l-arginine, theanine, and glutamic acid in real tea samples had been made possible using such a potentiometric sensor array. Subsequently, an amperometric e-tongue with noble metal working electrodes made into a flow-through sample-cell arrangement was used to differentiate among four varieties of tea; namely, two types of green tea, black, and oolong tea (Scampicchio et al., 2006). A mathematical model was developed from the e-tongue response and sensory analysis to predict the astringency of those tea samples. A potentiometric taste sensor array based on gate modified field effect transistors were used for estimation of caffeine and catechins in green tea (Chen et al., 2010). Good detection accuracies were obtained with multivariate calibration models. A novel e-tongue based on the principles of electrochemical impedometry was proposed by Bhondekar et al. (2010) for tea liquor. It was shown that by using such a system, chemical characterization of tea liquor is feasible in terms of various operating frequencies and the type of working electrodes. An e-tongue based on the principle of fluorometry has been proposed (Chang et al. 2010). The sample solutions were irradiated by UV light and the resulting intensity of fluorescence was detected to determine the concentration of tea amino acid and tannins for subsequent taste assessment of umami and astringency of real tea samples. However, the quality prediction of tea infusion using an e-tongue was first proposed by Palit et al. (2010a). The instrument was calibrated in terms of the human generated quality scores from 1 to 10 and it was used to classify different grades of tea based on the taste of tea infusion.

## 24.3 OBJECTIVES AND PHILOSOPHY

The objective of this chapter is to describe the application of a voltammetric e-tongue for the estimation of important biochemicals that affect the taste quality of black CTC tea. The two groups of compounds selected for our study are TF and TR. The quantification of TF fractions have also been explored because these are better quality indicators of tea. The significance of the work described in this chapter may be supported by the fact that the reference quality scores used for calibration of e-tongue data models in the previous work (Palit et al., 2010a) contained the uncertainties of human perceptions. It is therefore imperative to describe the quality of tea by e-tongue in terms of the concentration of TF and TR. The analysis steps leading to the detection method discussed shall also justify the applicability of e-tongue for quality estimation of tea samples in terms of chemical parameters that convey a better and consistent idea about quality. The sections that follow describe the e-tongue setup, and two case studies that illustrate the feasibility of e-tongue for this topic. Case study I discusses the estimation of total TF and TR in tea liquor with reference values determined by a UV-visible spectrophotometer. Case study II describes the estimation of four TF fractions along with total TF (calculated as the sum of all TF fractions present in tea liquor), where the reference values have been determined using high-performance liquid chromatography (HPLC).

## 24.4 THE VOLTAMMETRIC ELECTRONIC TONGUE SETUP

Fig. 24.2 describes various components of a voltammetric electronic tongue used in this study. The three major components of this e-tongue system are:

1. an electrode array acting as a transducer for sensing the chemicals in test sample
2. the hardware interface module cum measurement circuit
3. the software module residing in a personal computer

A working electrode array of five different noble metals—gold, iridium, palladium, platinum, and rhodium—was used in this study. The electrodes were circularly arranged around the reference electrode with a radius of 12 mm. This was done to reduce the effect of solution resistance as compared to the currents due to redox reactions on the total current response. A stainless steel counter electrode along with an Ag/AgCl reference electrode (saturated KCL, Gamry Instruments Inc., USA) was used.

A multielectrode potentiostat based on the three electrode principle was developed for applications with tea

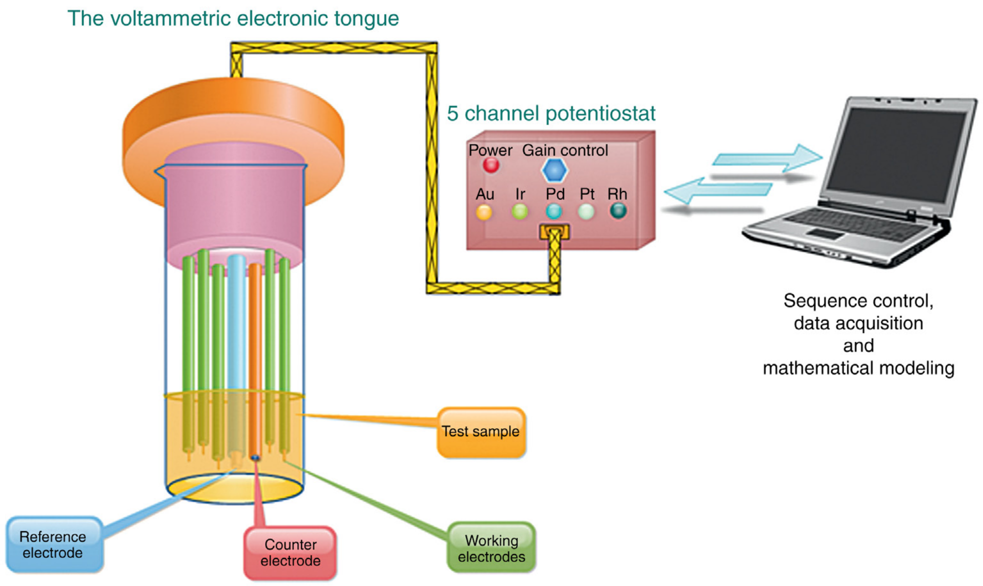

**FIGURE 24.2** **The working schematic of the electronic tongue and the component modules.**

samples. The pulse parameters (ie, pulse amplitude sequence and pulse width), and the circuit parameters (ie, gain and time constant of input filters) were systematically optimized after extensive experiments with tea samples.

The responsibility of the software module is the execution of sequence control steps for operating the hardware interface module and recording their measurements. The software module also performs the processing, presentation, and analysis of the measured data. The hardware interface module generates the control voltages specified by the software module, routes these voltage sequences to the electrochemical cell, and also measures the resulting electrical response from the electrodes. The data acquisition system performs the necessary analog to digital conversions and vice versa. It also generates the control voltages, while the associated electronic circuitry applies these voltages to the electrodes after necessary processing. The electrical response from the electrode array are processed and measured by the analog circuits and subsequently digitized by the data acquisition system before being forwarded to the software module for data analysis purposes.

## 24.5 CASE STUDY I: ESTIMATION OF TOTAL THEAFLAVINS AND THEARUBIGINS IN BLACK TEA (GHOSH ET AL., 2012)

The experiment started with the procurement of 46 different tea samples over 2 seasons (Apr.–May and Sep.–Oct.). The TF and TR contents of the samples were analytically determined using a UV–Vis spectrophotometer and the same samples were presented to the e-tongue for collection of electronic responses. The experimental steps are summarized in Fig. 24.3.

The tea liquor samples for collecting the response of e-tongue was prepared by boiling 200 mL of deionized water poured over 1 g of dry tea. The solution was allowed to brew for 10 min, after which it was stirred well to uniformly mix the extract with water. The samples were then presented to the e-tongue at room temperature. The potentiostat section of the e-tongue generated a large amplitude pulse waveform varying from +0.8 to −0.2 V in small user-defined steps of 100 mV that was applied to sample through the working electrodes connected one at a time. The working electrodes were selected sequentially by a switching circuit. The voltage equivalent of output current from the test sample was applied to the data acquisition card, where it was collected and stored for data analysis. At room temperature, 38 responses were recorded for each of the 46 tea samples. Thus, a data matrix of size 3470 × 1748 was obtained for data analysis. The complete large amplitude pulse voltammetric (LAPV) response waveform thus consisted of 3470 ($694 \times 5 = 3470$) data points.

The raw data set needs preprocessing for condensing the information content in terms of fewer data points and suitable conditioning of data for enhancing the performance of data analysis procedures. The preprocessing stages followed were mean-centering of each waveform and feature extraction using the sixth level wavelet transform with Haar as the mother wavelet (Palit et al., 2010b) to obtain 55 transformed variables. The data set after feature extraction became 55 × 1748. PCA score plots presented in Fig. 24.4 indicates clustering tendencies among

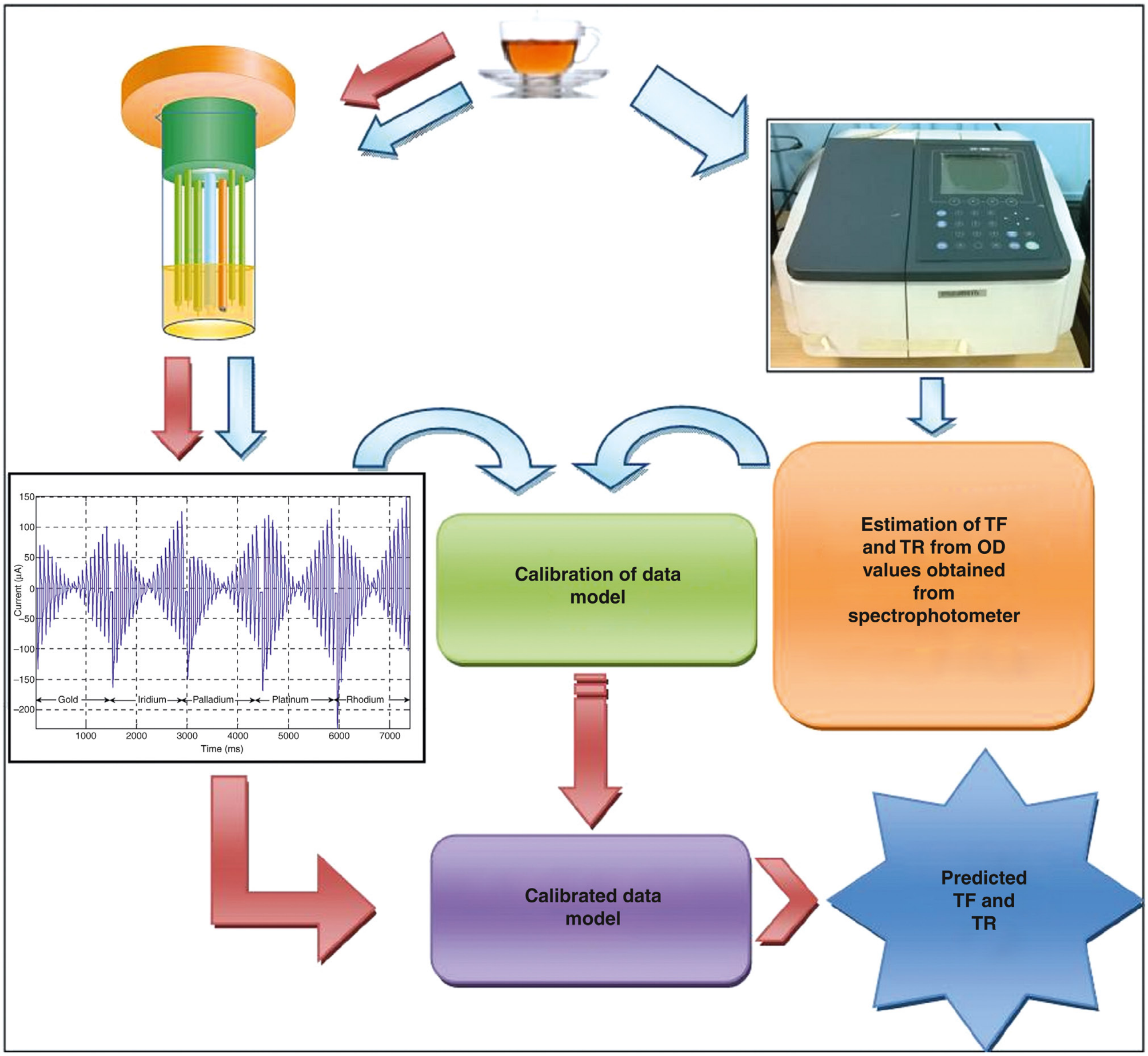
150
100
50
0
−50
−100
−150
−200
Current (µA)
Gold
Iridium
Palladium
Platinum
Rhodium
1000
2000
3000
4000
5000
6000
7000
Time (ms)
Calibration of data model
Estimation of TF and TR from OD values obtained from spectrophotometer
Calibrated data model
Predicted TF and TR

**FIGURE 24.3** **The steps for the prediction of TF and TR.**

the samples responses with similar TF and TR values. The colors of markers indicate the observations corresponding to tea samples with the concentration of TF/TR within a specified range as presented in the plot legend.

The first two principal component directions, PC1 and PC2, cumulatively contribute 93% variance, while that for TR has been found to be 95.8%. It may be deduced from the PCA plots that there is a complex and nonlinear interaction between the e-tongue response and the TF, TR content. The use of nonlinear, powerful and efficient models is essential not only to differentiate among the clusters but also to develop a regression model from these localized distributions of data.

In order to develop the prediction models, three data modeling techniques were implemented using partial least squares regression (PLSR) (Wold et al., 2001), support vector regression (SVR) (Haykin, 2001), and back-propagation neural networks (BPNN) (Haykin, 2001) with two types of weight optimization algorithms, gradient descent and back propagation neural network (GD-BPNN) and scaled conjugate gradient (SCG-BPNN) (Moller, 1993). A large number of parameter optimization steps for all the data modeling techniques over repeated independent trials revealed that a three layer BPNN trained by a SCG-BPNN algorithm produced best results. In order to evaluate the efficacy of

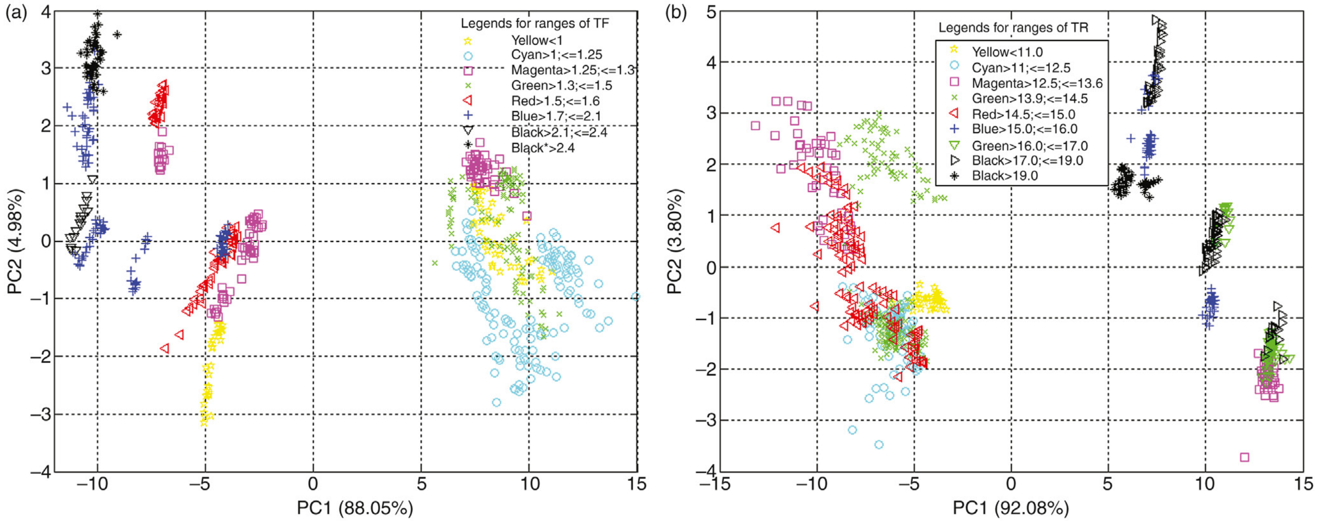

**FIGURE 24.4** Plots of electronic tongue responses showing clusters with values of (a) TF and (b) TR.

developed models, the following performance parameters were calculated during the testing phase of model development:

1. CF: correlation factor between predicted and actual value
2. WPA: worst prediction accuracy
3. MPA: mean prediction accuracy
4. MSEP: mean square error for prediction
5. SDPE: standard deviation for prediction error

The results obtained with best models developed using SCG-BPNN for TF and TR are presented in Table 24.1.

It may be stated at this point that the SCG algorithm was chosen as it was reported to be effective for large scale optimization (Moller, 1993) as result of which it develops more efficient and accurate architectures. It may be observed that the prediction model developed for TF produced a correlation factor of 0.98 along with minimum and mean accuracy of 78.72 and 96.5%, respectively with respect to the spectrophotometric estimations. Regarding the development of prediction model for TR, the minimum and mean accuracy was found to be 87.99 and 97.93%, respectively. The developed model produced better mappings with correlation factors of 0.98 and 0.97 for TF and TR, respectively.

The above results indicate that a voltammetric e-tongue could be applied to determine the approximate TF and TR content in tea. However further researches are required to identify and predict the concentrations of other biochemical compounds affecting the quality of tea, improve the separation among responses by optimizing the selection of electrodes and modify the experimental procedures for optimization of experimental conditions for better sensing performance.

## 24.6 CASE STUDY II: ESTIMATION OF THEAFLAVIN FRACTIONS FROM ELECTRONIC TONGUE RESPONSE

In some cases total TF levels do not correlate with the sensory analysis. This has led to the research regarding the TF fractions. It was found that the TF fractions correlate with sensory analysis better than total TF. The TF composition of black tea is dominated by four fractions:

1. simple theaflavin (TF)
2. theaflavin-3-gallate (TF-3-g)
3. theaflavin-3′-gallate (TF-3′-g)
4. theaflavin-3,3′-digallate (TF-3,3′-dg)

**TABLE 24.1** Prediction Results of TF and TR by Voltammetric Electronic Tongue

| Target | Architecture | CF | WPA (%) | MPA (%) | MSEP | SDPE | $R^2$ |
|---|---|---|---|---|---|---|---|
| TF | 55-55-1 | 0.98 | 78.72 | 96.50 | 0.005 | 0.07 | 0.96 |
| TR | 55-45-1 | 0.97 | 87.99 | 97.93 | 0.17 | 0.41 | 0.94 |

These TF fractions are produced due to the oxidative dimerization of simple (dihydroxilated) catechins and gallo (trihydroxilated) catechins as shown in Eqs. (24.1)–(24.4):

$$\mathrm{EC} + \mathrm{EGC} \rightarrow \mathrm{TF} \tag{24.1}$$

$$\mathrm{EC} + \mathrm{EGCG} \rightarrow \mathrm{TF\text{-}3\text{-}g} \tag{24.2}$$

$$\mathrm{ECG} + \mathrm{EGC} \rightarrow \mathrm{TF\text{-}3'\text{-}g} \tag{24.3}$$

$$\mathrm{ECG} + \mathrm{EGCG} \rightarrow \mathrm{TF\text{-}3,3'\text{-}dg} \tag{24.4}$$

The galloyation of TF affect the astringency taste of the molecules in increasing order. Thus, the TF digallates containing two gallate groups are most astringent followed by TF monogallates and simple TF. It was found that the astringency ratio of TF-3,3′-dg:TF-3-g:TF-3′-g:TF is 6.4:2.22:2.22:1 (Owuor et al., 2006). But the astringency contributions of individual TF do not contribute in terms of their micro-molecular amounts but according to the ratio of their total activity values (TAV) (Scharbert et al., 2004b). The TAV is defined as the quotient of the actual concentration in tea and the human taste detection thresholds in water. The TAV figure thus correlates more aptly with the sensory analysis. The estimation of the TF fractions are also important, considering the fact that the relative amounts of these fractions vary according to geography (McDowell et al., 1991). For example, in Kenyan black tea the amount of simple TF > TF-3-g > TF-3,3′-dg > TF-3′-g (Owuor and Obanda, 1995). On the other hand, Assam black tea produces highest amount of TF-3,3′-dg, while simple TF is produced in a highest amount in Ceylon tea (Scharbert et al., 2004a). Estimation of TF fractions may thus explain the disparity between sensory and biochemical analysis leading to the development of a general metric regarding tea quality encompassing geographical variations.

The objective of this section is to investigate the effect of these fractions on the e-tongue response and whether a voltammetric e-tongue can be used to estimate the concentration of TF fractions.

As a part of experimental procedure, total 40 tea samples were collected over 2 seasons (Oct.–Nov. 2013). The concentrations of four TF fractions present in these tea samples [simple TF, TF-3-g, TF-3′-g, TF-3,3′-dg, and total TF (sum of estimated fractions)] were determined in milligram per gram (dry weight) using HPLC analysis following a standard method (Sabhapondit et al., 2014) and the same samples were presented to the e-tongue for the development of calibration models.

The tea liquor samples for e-tongue were prepared by boiling 150 mL of ultrapure water poured over 0.9 g of dry tea. The solution was allowed to brew for 10 min, after which it was stirred well to uniformly mix the extract with water. The samples were filtered using nonabsorbant cotton and allowed to cool down to room temperature. The potentiostat section of the previously mentioned e-tongue was used to apply a large amplitude pulse waveform varying from −0.9 to +0.9 V in small user-defined steps of 0.1 V to the sample through one working electrode at a time. Then 1488 data points were collected at the sampling rate of 1 KHz from each of the five electrodes. The complete large amplitude pulse voltammetric (LAPV) response waveform thus consisted of 7400 (1480 × 5 = 7400) data points. Twenty-five replicated responses were recorded for each of the 40 tea samples. Thus, a data matrix of size 7400 × 1000 was obtained for data analysis. In order to perform the feature extraction step, the following features were selected from each pulse response segment:

1. the current value at the onset of the response pulse
2. current value at the end of the response pulse
3. the time constant of pulse roll-off from initial to final current value

Thus, from the whole response spanning 7400 sample points, only 540 data points were obtained.

In this study, the calibration models were developed using PLSR method. The model performance was tested using the leave-one-out cross-validation (LOOCV) method where the parameter value of an unknown sample was obtained from the PLSR model calibrated by the parameter values of the remaining 39 samples. Five different models were developed corresponding to four TF fractions and a total TF. The optimum number of PLS components were determined by repeated trials. The results of LOOCV are summarized in Table 24.2.

**TABLE 24.2** Summary of LOOCV Results for Prediction of TF Fractions Over 40 Tea Samples Using a Voltammetric Electronic Tongue

| Biochemical Parameters | Average Accuracy Over 40 Samples (%) | Optimum Number of PLS Components |
|---|---|---|
| Simple TF | 87.08 | 6 |
| TF-3-g | 84.02 | 26 |
| TF-3′-g | 90.96 | 22 |
| TF-3,3′-dg | 91.77 | 22 |
| Total TF | 90.12 | 24 |

It may be observed that the average accuracy of prediction is above 85% except for TF-3-g, which is 84%. The most notable results have been obtained nearly at 92% for TF-3,3′-dg. This result is significant, considering the fact that TF digallates are most abundant in Assam CTC tea, followed by TF-3′-monogallates. The prediction accuracy concerning simple TF is also encouraging, considering the fact that Darjeeling, Ceylon, and Kenyan tea have greater amounts of simple TF followed by TF-monogallates. It may thus be expected that e-tongue responses may be influenced by the TF digallates and its higher contribution in overall astringency. This experiment again proves that total TF is quantifiable by an e-tongue even at the sample level cross-validation procedure.

## 24.7 CONCLUSIONS

In this chapter, a voltammetric electronic tongue based on an array of noble metal electrodes has been described for the detection of quality affecting biochemicals in black tea. Two groups of compounds— the TF and the TR—contribute significantly to the taste and appearance of the tea liquor and the e-tongue described here has been shown to detect not only the amount of these two groups of compounds but also their fractions using suitable data processing steps. To validate this point, the experiments were carried out in two steps. First, the response of the e-tongue was correlated with the spectrophotometer where total TF and TR concentrations were determined. The prediction models were developed with neural networks from the e-tongue response. It may be observed that the predictions of the developed data models agree with the spectrophotometric estimations at accuracy of over 95%. In the second part, the response of e-tongue was correlated with the HPLC instrument. The HPLC instrument was used to determine the concentrations of four TF fractions. The data model developed from the e-tongue response again agreed with the actual estimations by 90% accuracy. Interestingly, the best accuracy has been obtained for the prediction of TF digallate and TF monogallate indicating the dominant effect of astringent compounds in the modeling performance. The results positively imply the feasibility of applying the described methodology using even a noble metal electrode array for the stated purpose. Further research may be carried out toward the development of customized electrodes sensitive to the target compounds in order to improve the modeling performances, propeling the electronic tongue technology toward cheap, objective, consistent, and rapid quality analysis of black tea.

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## Chapter 25

# Drinking Water Analysis Using Electronic Tongues

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## 25.1 INTRODUCTION

### 25.1.1 Taste Sensing in Nature

Gustation and olfaction are the natural senses used to classify the chemical composition changes in the environment of living organisms, which might be vital information about the danger or the quality of food. The capabilities of these analytical systems has been therefore important to the evolution of life on Earth. Both the sensations of smell and of taste deal with a hard analytical task: to distinguish an enormous variety of chemicals. The goal to classify a large number of different odorants or taste molecules with relatively few sensitive elements has been brilliantly accomplished by the integration of receptors with broad overlapping specificities, and successive signal processing by pattern recognition in the brain, yielding very broad quantitative and qualitative analytical information.

Optimized by evolution, the gustation system of most living organisms can identify chemical compounds (taste molecules) of diverse molecular structures with high sensitivity and accuracy. The taste buds are found in thousands on the human tongue with a few on the soft palate, the inner surface of the cheek, pharynx, and epiglottis of the larynx (Deisingh et al., 2004). A single taste bud contains up to 100 taste cells, responsible for all 5 basic taste sensations, that is, sweet, sour, bitter, salty, and umami (savory or glutamate), containing the corresponding receptors. The reversible binding of taste molecules to receptors at the taste cells (Adler et al., 2000; Matsunami et al., 2000) as a gustation primary process produces an input signal, which in turn produces a cascade of transduction events with information output toward perception in the gustatory cortex of brain (Scott, 2005). The full perception of human taste is a merging of five basic tastes with a descriptive taste contribution from the olfactory information of the food aroma. Different taste receptors have cross-reactivity toward the same taste molecules (Zhang et al., 2003), ensuring a platform of sensitive elements with distributed semi-specificity with the same functional principle as for the olfactory system.

### 25.1.2 Artificial Taste Sensing

The understanding of the process of human olfaction and gustation accomplished in the past few decades has inspired the ambitious idea to mimic the natural sensing systems in order to elaborate analytical systems with similar performance as the natural predecessors. This has led to a new strategy in chemical analysis; instead of specific recognition-based quantification, it is in many applications advantageous to determine quality parameters. The approach overcomes the disadvantages of sensors operating due to specific chemical recognition, which include isolation of a recognition element responsible for the high specificity and the integration with an appropriate and effective signal transducer, while maintaining the recognition activity during and after device assembly. The avoidance of a very specific recognition element led to the creation of a robust, high throughput and versatile platform for chemical analysis. This has been achieved in analogy with the human senses (Fig. 25.1) by the creation of an array of semiselective sensors with overlapping specificities and with differentiated responses toward different analytes of complex samples, accompanied with signal processing and pattern recognition, which enables a rational decision.

The complex signal obtained from the array is interpreted with multivariate data analysis (MVDA) (Gouma et al., 2004; Jurs et al., 2000; Winquist et al., 2004), that is, different types of algorithms as mathematical tools to extract useful information from large data sets. Some of the most common are principal component analysis (PCA), clustering systems such as hierarchical cluster analysis (CA), linear discriminant analysis (LDA), partial least squares (PLS), and artificial neural networks (ANN).

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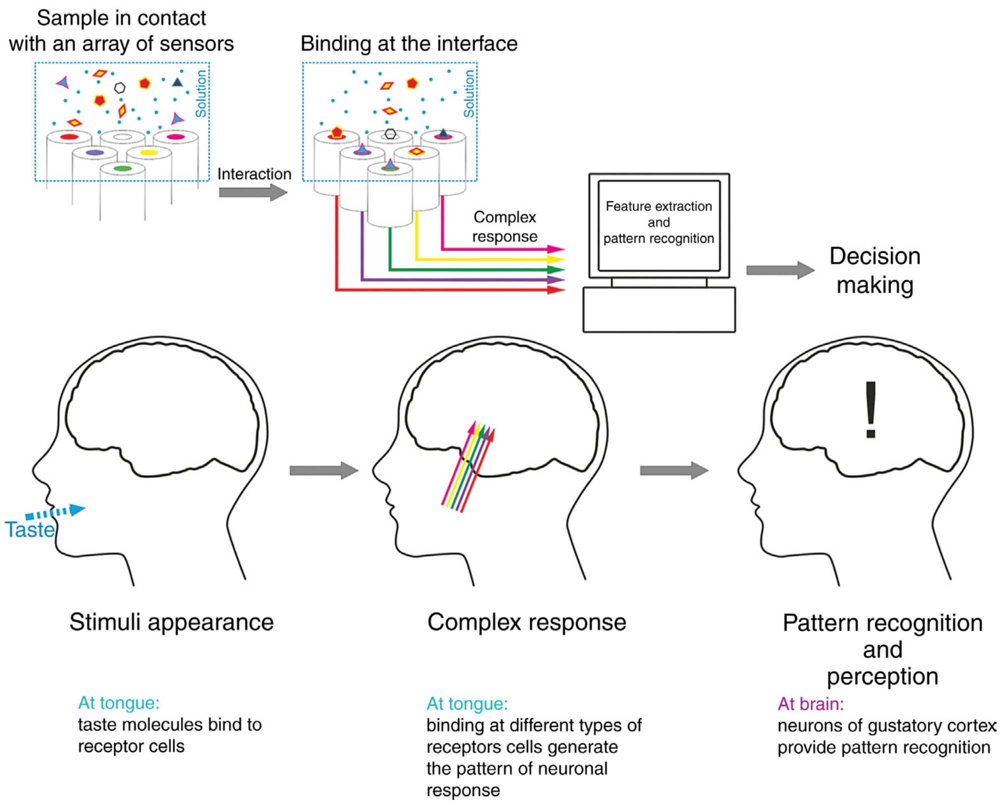
Sample in contact with an array of sensors
Binding at the interface
Solution
Solution
Interaction
Complex response
Feature extraction and pattern recognition
Decision making
Taste
!
Stimuli appearance
Complex response
Pattern recognition and perception
At tongue:
taste molecules bind to receptor cells
At tongue:
binding at different types of receptors cells generate the pattern of neuronal response
At brain:
neurons of gustatory cortex provide pattern recognition

**FIGURE 25.1** Analogy of human gustation and artificial taste sensing technology.

## 25.2 THE E-TONGUE

Two strategies have been utilized for the development of analytical systems similar to olfaction and gustation. The so-called taste sensor (Taniguchi et al., 1999; Toko, 1996) follows the human gustation and mimics the five basic tastes. Alternatively, the e-tongue (Di Natale et al., 1996; Winquist et al., 1997) and the electronic nose (e-nose) (Gardner and Bartlett, 1994; Winquist et al., 1993) mimic the sensing process and analyze the chemical properties of the sample in liquid and gaseous phases, respectively, which is more equivalent to the taste impression of food entering the mouth as the combination of taste and smell. The chemical properties assessed by e-tongue and e-nose do not necessarily correlate with the human perception. In this chapter, the e-tongue will be considered.

Artificial sensation systems consist of three integrated parts: (1) a sensing unit, which transforms chemical information from the interaction reaction as an input signal to an electronic read-out output, (2) a signal-processing unit, which collects the responses during the measurements and supplies preprocessed signal to (3) a pattern recognition system, which compares the processed response with a library of profiles of known substances.

Decoding the chemical energy of the interaction between the sensing unit and the analytes into a primary signal output, the array of sensing elements determines the performance of the whole analytical system. E-tongue instruments rely on available analytical technologies operating in the liquid phase. The most common ones are based on electrochemical techniques such as voltammetry, potentiometry, and conductometry, which require the use of electrodes in the liquid phase in order to establish a measurement circuit. The currents passing through the electrodes or the potential difference between them are affected by analyte reactions, which creates the primary read-out signal of e-tongues based on ion selective electrodes (Di Natale et al., 1997) or voltammetry (Winquist et al., 1997). Conductivity changes of conducting polymers due to the interactions with analyte molecules fabricated onto electrode surfaces were also utilized for e-tongue elaboration (Sangodkar et al., 1996). An array of ion-selective field effect transistors (ISFET) with overlapping selectivities has been utilized in a commercial e-tongue instrument, for example, Astree (Alpha MOS). Optical methods based on measurement of light absorbance (Fries et al., 2011) or fluorescence (Sohn et al., 2005; Szurdoki et al., 2000) have also been utilized for e-tongue instrument elaboration. Shifts of resonance frequencies of piezoelectric crystals as a result of interaction between the membrane and an analyte in a liquid media was utilized for e-tongues (Rabe et al., 2003). The use of surface plasmon resonance for differential sensing in liquid media was also reported (Nanto et al., 2002).

The subsequent analysis of the data, obtained as the primary response of the sensor array, starts with a preprocessing step of drift compensation and response normalization (Dymerski et al., 2011). The choice of preprocessing, from the available techniques of statistical analysis, depends on the type of the system and the data obtained. The first approach for the classification of samples with significant difference is usually a graphical presentation of the response data in different diagrams (polar diagram, histogram, etc.) (Dymerski et al., 2011). More advanced methods of analysis are based on statistical handling of data for feature extraction and finding of the most important parameters. There are many multivariate methods that can be utilized, for example, PCA, PLS, CA, canonical correlation analysis (CCA), discriminate function analysis, multidimensional scaling, and LDA (Dymerski et al., 2011; James et al., 2005). Alternatively, the data processing systems based on the principles of the human nervous system, that is, ANN, constructed as hardware or software models can be employed for the data analysis where the distribution is completely unidentified (Dymerski et al., 2011; Haugen and Kvaal, 1998).

The e-tongue can be used for the detection of dangerous or poisonous compounds, which is one of the main concerns of drinking water analysis. Moreover, e-tongue detection is also elaborated as a platform for robust monitoring of quality parameters in general, that is, a global quality monitoring system.

## 25.3 DRINKING WATER QUALITY

Sustainable and safe supplies of drinking water are worldwide challenges with the potential to improve the quality of life of billions of people (UNESCO, 2012). Improvement of water resource management, increase of the access to safe drinking water, and basic sanitation are critical for the achievement of the goals to reduce child mortality, improve maternal health, and reduce the burden of waterborne diseases (United Nations, 2012). From a societal point of view, the problem of supplying secure and safe drinking water has been given increasing attention recently, both on national and international levels. The EU Water Framework Directive (The European Union, 2000), being implemented by all EU member states, addresses the management and protection of water supplies.

In general, the greatest microbial risks are associated with ingestion of water that is contaminated with human or animal (including bird) feces. Feces can be a source of pathogenic bacteria, viruses, protozoa, and helminthes (World Health Organization, 2008). Fecal-related substances, such as domestic sewage water, are therefore good markers for pathogenic microorganisms in the drinking water. Drinking water can also be contaminated with raw water for natural reasons or by antagonistic purpose (terrorist threats), which opens the concept of drinking water safety and security.

Even developed countries have drinking water concerns—for example, a recent sewage water contamination of a drinking water supply in Östersund, Sweden, 2010; the erroneous connection of the sewage water pipe to the drinking network in the city of Nokia, Finland, 2007; and the accidental release of large amounts of alumina sulfate in Camelford, United Kingdom, 1998—which in all cases resulted in thousands of affected people and considerable costs for the society. It has been concluded (Lindberg et al., 2011) that apart from the suffering of the 27,000 people who sickened in Östersund, a cost for the society of 24 million Euro can be estimated. Pathogenic microorganisms causing waterborne outbreaks by drinking water contamination include bacteria (*Campylobacter* and pathogenic *E. coli*) (Szewzyk et al., 2000), viruses (*Norovirus*) (Maunula et al., 2005), and protozoa parasites (*Cryptosporidium* and *Giardia*) (Mackenzie et al., 1994).

## 25.4 THE E-TONGUE FOR DRINKING WATER ANALYSIS

Being a complement to e-nose instruments' measuring in the gas phase, the e-tongue sensing technology is highly effective for applications where it is more advantageous to measure directly in the liquid phase, for example, when the ionic composition changes or redox active nonvolatile compounds are present. The e-tongue sensing technology is of special relevance for the detection of water pollution (Di Natale et al., 1997) and a variety of electrochemical and optical methods have been developed for this application. Electrochemical methodology opens the possibility for operation in high turbidity media for raw or surface water analysis, which is an advantage when comparing with optical methods. Thus, monitoring of both water supplies and drinking water has been extensively developed by e-tongue technology (Riul et al., 2010).

### 25.4.1 Monitoring of Tap Water Quality

Drinking water quality can thus be monitored with e-tongue instruments. Full water works processes can be monitored with voltammetric e-tongues for the detection of quality changes (Lindquist and Wide, 2001; Winquist, 2008; Winquist et al., 2004). The raw water, which is the untreated water, is taken either from surface (river or lake) or from ground water (natural or drilled wells). The raw water normally has significant color changes due to the seasonal and weather variations. At a first stage of drinking water production, chemical precipitation by aluminum sulfate is performed to get rid of small particles. From the first stage of production, the water is transported to a sand filter operating with a fast flow (so-called fast filter) to remove the rest of the particles. Further processing is performed at a slow flow rate through a biological active slow sand filter to get rid of germs and flavors. Finally, after the addition of sodium hypochlorite and sodium hydroxide to kill the rest of the germs and to adjust the pH value, the water is transported out to the distribution network. The whole drinking water production has been monitored with a voltammetric e-tongue based on four metal electrodes (gold, iridium, platinum, and rhodium) and followed by the classification by PCA (Krantz-Rulcker et al., 2001). A similar strategy of primary signal collection has been utilized for the e-tongue developed with subsequent data compression by wavelet transform together with different wavelet selection algorithms and utilized for drinking water monitoring (Artursson and Holmberg, 2002). A similar concept has also been developed for field tests of drinking water quality monitoring in a flow (Lindquist and Wide, 2004).

A fast drinking water quality assessment has been implemented with e-tongue measurements (Iliev et al., 2006) based on voltammetric primary signal read-out on gold and platinum wires with respect to a stainless-steel counter-reference electrode. A fuzzy clustering technique was used for the classification of good and bad quality samples from a training set. Further online classification of the measured response for unknown samples to three clusters (good, uncertain, and bad) yielding a decision about the water quality to the user in a simple way in the form of “traffic light” signals. The developed system showed satisfactory performance (Table 25.1) with correct classification for 90% of the drinkable water (green light), 100% of the undrinkable river water (red light), and 87% of the boiled river water (bad quality drinkable, yellow light). The undrinkable mixtures of river and tap waters were between the red (79%) and yellow (21%) clusters. The developed monitoring platform revealed a satisfactory performance since none of the undrinkable water samples were classified as drinkable.

Quality variations of the raw water may be due to high amounts of microorganisms or industrial contaminants. These disturbances accompanied with unpleasant odor and/or taste do generally not possesses a health risk, if the raw water is processed properly in the drinking water production plant. Mechanical damage of the inner coating of pipes of the distribution net may cause discoloring and unpleasant taste, which in most cases is harmless for the health. Contamination of the drinking water may also occur due to mixing with water from an external source as a result of natural causes, such as an effect of flooding due to heavy rain showers, by industrial accidents such as the erroneous connections in the distribution network or for antagonistic purposes. In Camelford (UK), large levels of alumina sulfate were accidentally added to the drinking water in 1998 (David and Wessely, 1995), leading to nausea, vomiting, and other symptoms. In Tel Aviv (Israel), ammonia was spilled into the main waterline in 2001 (Winston et al., 2003), causing high pH and turbidity levels in the drinking water. Various model contaminations of drinking water (NaCl, $NaN_3$, $NaHSO_3$, NaOCl, ascorbic acid, and yeast suspension) have been used and classified with voltammetric e-tongues with PCA and PLS data processing (Winquist et al., 2011).

Remote monitoring of drinking water quality has been implemented with an e-tongue network (Eriksson et al., 2011) utilizing the voltammetric primary signal from an array of three working electrodes (gold, platinum, and rhodium) with respect to a stainless-steel counter-reference electrode. Potential pulses with different amplitudes were applied onto the working electrodes, which resulted in the appearance of current transients containing analytical information about the monitored media (Fig. 25.2). In particular, the size and shape of the obtained current transients were affected by the pulse amplitude and the concentration of the substances undergoing electrode reactions and of the ions in the media as well as their diffusion characteristics. Signal processing was carried out by feature extraction followed by PCA with a remote personal computer via Internet, which opens the possibility for remote monitoring of drinking water and surface water quality. A probability density function was utilized as an evaluation criteria to distinguish different signal anomalies due to water pollution from the normal (harmless) variations in the drinking water characteristics due to natural variations in temperature, flow velocity of the water, pH, turbidity, and residual chlorine. The strongest impact came from the temperature variations, which were taken into account by introducing a temperature sensor.

**TABLE 25.1** Results of the Classification of Water Samples.

| Water Sample | Green (%) | Yellow (%) | Red (%) |
|---|---|---|---|
| Tap (drinkable, good quality) | 90 | 7 | 3 |
| River (undrinkable) | 0 | 0 | 100 |
| Boiled river (drinkable, bad quality) | 10 | 87 | 3 |
| Mixture of river and tap (undrinkable) | 0 | 21 | 79 |

Source: Reproduced from Iliev et al. (2006), with permission from Elsevier.

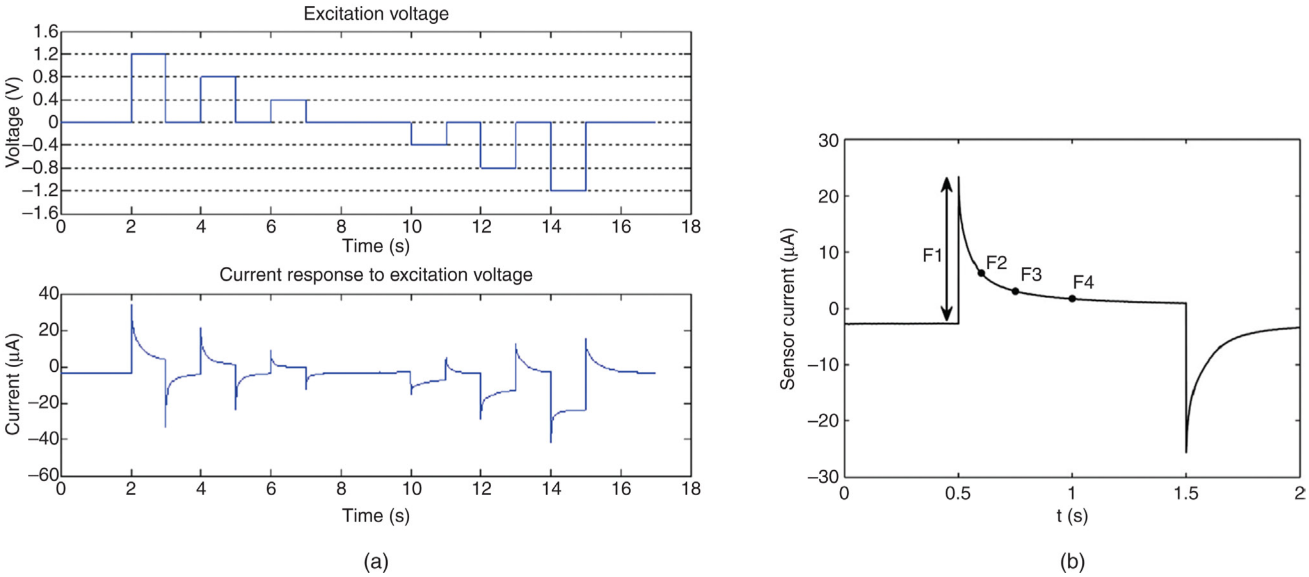

**FIGURE 25.2** (a) Excitation voltages (top) and resulting current responses (bottom) in a typical measurement of noncontaminated drinking water in a pilot system with similar properties as the real drinking water distribution system. (b) Current response due to one of the voltage pulses in (a). The positive current transient is due to the sudden increase in voltage and the negative current transient to the sudden decrease. The exact shape of the current transients depends on several parameters, such as the type and concentration of substances added to the drinking water. F1–F4 are examples of features that are extracted from the sensor signals. *(Reproduced from Eriksson et al., 2011, with permission from Elsevier.)*

The detection of drinking water contaminations was successfully performed by an algorithm without any confusion by the temperature variations (Figs. 25.3 and 25.4). The elaborated e-tongue instrument provided a fast alarm and a network of such sensors can be effectively utilized for the determination of the contaminated part of the distribution network, for the forecast of the pollution spreading and for the identification of the location of the pollution source.

Complex conductivity measurements have been used for e-tongue monitoring of drinking water quality and the classification of potable and nonpotable water samples (Oliveira et al., 2013). An array of six interdigitated microelectrodes

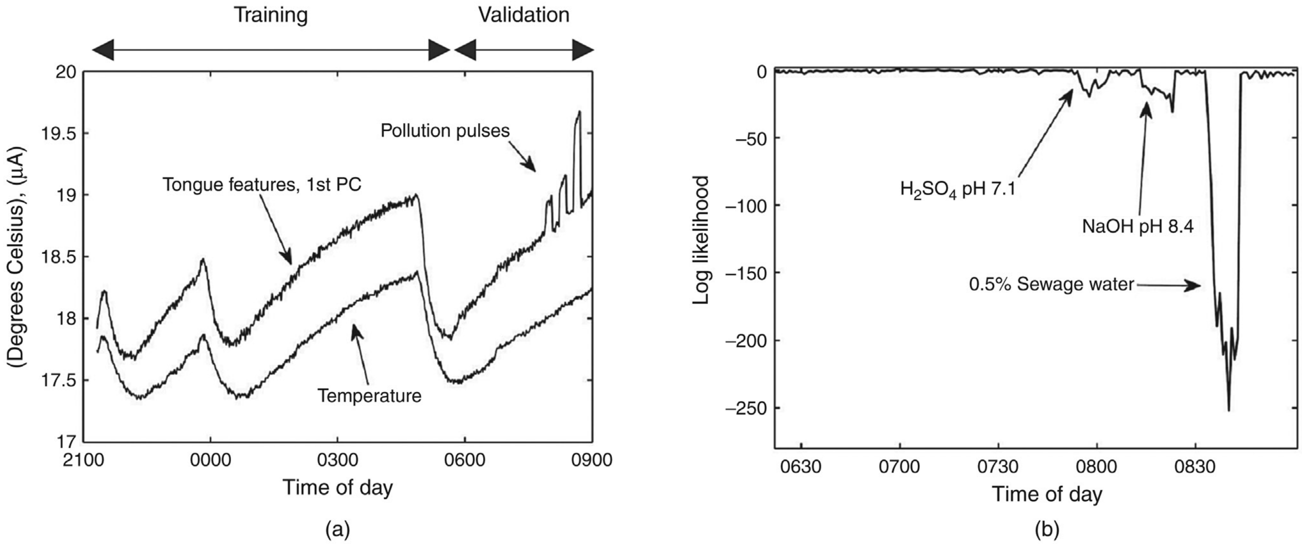

**FIGURE 25.3** (a) The first principal component (PC) of extracted features of the rhodium electrode signals and temperature measurement data. At the end of the measurement, three pulses of different pollutants are added: $H_2SO_4$ (changing the pH from 8.2 to 7.1), NaOH (changing the pH from 8.2 to 8.4), and, finally, 0.5 % sewage water. (b) The first 9 h from the 12 h data set of (a) *(the training part)* have first been used to train a statistical model for change detection. The model is then applied to the final 3 h of the data set of (a) *(the validation part)*. Since the pollution measurements do not fit the model well, they appear very unlikely, and therefore deviate strongly from the background level. *(Reproduced from Eriksson et al., 2011, with permission from Elsevier.)*

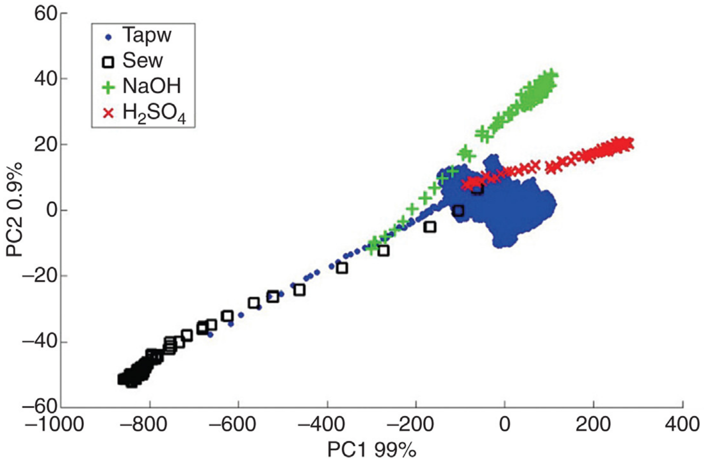

**FIGURE 25.4 Classification of the different pollutants added to the drinking water with PCA.** Drinking water *(blue)* is the reference medium. Addition of sewage water (0.25%, *black*) results in data taking off in a completely different direction than those of NaOH [0.3% (pH increases from 7.7 to 9.4), *green*] and $H_2SO_4$ [0.4 % (pH decreases from 7.7 to 7.0), *red*]. *(Reproduced from Eriksson et al., 2011, with permission from Elsevier.)*

modified with a film of poly(lactic acid)/carbon nanotube composites of different ratios of components were used for complex conductivity measurements as a primary signal of the instrument, further classified with PCA. The drinking water samples were successfully classified from water samples contaminated by heavy metal ions and traces of pesticides.

## 25.4.2 Mineral Water Analysis

An array of 29 potentiometric sensors of different materials and data processing with PCA has been combined for the construction of an e-tongue (Legin et al., 1999). The classification of seven Italian commercial mineral waters was successfully achieved. Moreover, the quantification of some components of mineral waters has been done with e-tongue. Contamination with a strawberry, as a model of organic matter, was detected with the developed instrument.

A disposable array of all-solid-state ion selective electrodes (ISE) has been developed by screen-printing with carbon ink doped by an inorganic redox compound (Prussian blue) with subsequent modification by polymer membranes of different selectivities toward cations and anions (Lvova et al., 2002). Potentiometry was used as the primary signal read-out of the e-tongue. Classification of tap and commercial drinking waters in a flow cell was done with PCA, PLS, and principal component regression. A taste map for commercial drinking waters has furthermore been developed.

An array of nine nonspecific all-solid-state ISE for different cations has been employed as a platform for e-tongue elaboration (Gallardo et al., 2005) with PCA processing. Operating in both batch and flow injection modes, the instrument was trained by a variety of solutions of known compositions. Satisfactory classification was achieved for 23 commercial mineral waters (Fig. 25.5).

An array of five metal electrodes (Au, Ag, Pt, Cu, and Zn) has been utilized for the construction of a potentiometric e-tongue with signal processing by PCA (Labrador et al., 2009). The instrument has been used for the qualitative and quantitative assessment of the concentrations of chloride, sulfate, and bicarbonate anions in eight mineral waters of different geographical origins. The training step was performed with solutions of pure salts, their mixtures, and some mineral water samples. Quantitative prediction was achieved by utilizing PLS.

An array of six independent ion selective field effect transistors has been developed on a single chip and utilized as an e-tongue for the classification of commercial drinking water by means of PCA (Moreno i Codinachs et al., 2008). Simultaneous read-out of multiple sensors was enabled by electrical isolation of the devices by trenches and by *p-n* junctions. Selectivity toward different ions was established by modification of the sensors with different organic membranes.

An array of different ISE has been utilized for the fabrication of an e-tongue with parallel statistical analysis by PCA and discriminate function analysis. A successful classification of Tunisian waters was achieved (Sghaier et al., 2009).

A potentiometric e-tongue has been developed on an array of ISE (Men et al., 2009). A primary signal was optimized by independent component analysis and LDA. A learning vector quantization model was utilized for the classification of five samples of mineral water. It was shown that the proposed classification approach surpassed the performance of the traditional self-organizing map algorithm.

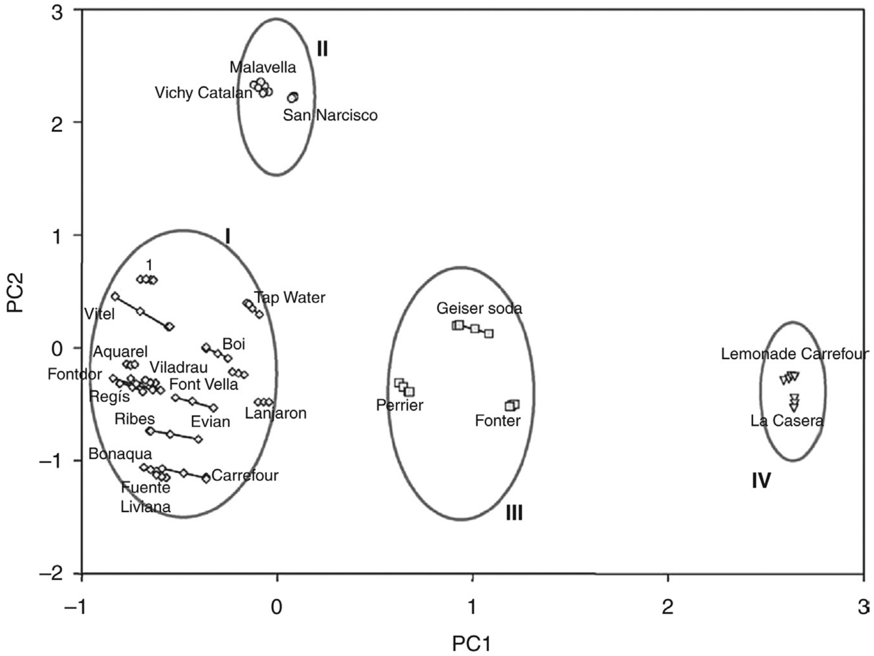

**FIGURE 25.5 PC1 versus PC2 scores plot of the PCA performed to data from mineral waters.** Groupings are *(I)*, mineral waters; *(II)*, sparkling mineral waters; *(III)*, added $CO_2$ mineral waters; and *(IV)* lemonades. *(Reproduced from Gallardo et al., 2005, with permission from Elsevier.)*

Mineral, spring, and tap water samples from different geographic origins has been classified by a commercial potentiometric e-tongue (Alpha Astree II, Alpha MOS, France) and compared with the results of a sensory panel evaluation and chemical analysis (inductively coupled with plasma atomic emission spectroscopy and ion chromatography) (Sipos et al., 2012). PCA and hierarchical cluster analysis as unsupervised methods and LDA as a supervised technique were used for the statistical analysis. A reliable classification was not achieved with any of the utilized techniques for different water samples from the same geographical origin. In particular, waters sold under different brand names but originating from the same region could not be distinguished with the aforementioned set of advanced analytical techniques. On the other hand, water samples obtained from different regions were effectively classified. Simultaneous application of analytical, e-tongue, and sensory techniques showed good correlation in the data clustering. A collective use of these different technologies is recommended for quality control of food products.

The cost-effective technology of screen-printing has been utilized for the fabrication of a potentiometric e-tongue with an array of twelve electrodes developed from conducting printable inks (based on $RuO_2$, C, Ag, Ni, Cu, Au, Pt, and Al) (Martinez-Manez et al., 2005a). The classification of five natural Spanish waters, tap, and osmosized waters has been achieved with a 93% success rate with PCA and ANN utilizing the so-called adaptive resonance theory for prediction of subsequent steps from the prior actions (fuzzy ARTMAP) (Figure 25.6). Further development of this concept has led to the elaboration of a portable e-tongue on an array of 18 potentiometric sensors developed by screen-printing (Garcia-Breijo et al., 2011). Five Spanish natural waters, sparkling water, and tap water has been studied. The data analysis system consisted of a pattern recognition algorithm implemented on a microprocessor system (Microchip PIC18F4550). The utilized data analysis algorithms allow fast, real-time operation on a portable instrument with a limited amount of memory, with high accuracy of classification. Only a few pattern recognition algorithms can fulfill each of these requirements and of these three pattern recognition algorithms were used. (1) Multilayer feed-forward (MLFF) is the most popular type of ANN and is based on three layers of neurons (input, hidden, and output). It requires a training stage, where the weight of each neuron is defined, followed by a validation stage. (2) Fuzzy ARTMAP network (see the previous description). (3) Linear discriminant analysis (LDA) is a probabilistic parametric classification technique maximizing the variance between categories and minimizing the variance within categories, by means of a data projection from a high- to low-dimensional space. Different techniques of training were employed for all three algorithms implemented on the microchip in order to obtain the optimum architecture for the network. All algorithms were compared by their recognition rates for drinking water classification. MLFF, fuzzy ARTMAP, and LDA showed recognition rates of 76.2, 76.2, and 82.5%, respectively. The recognition rates

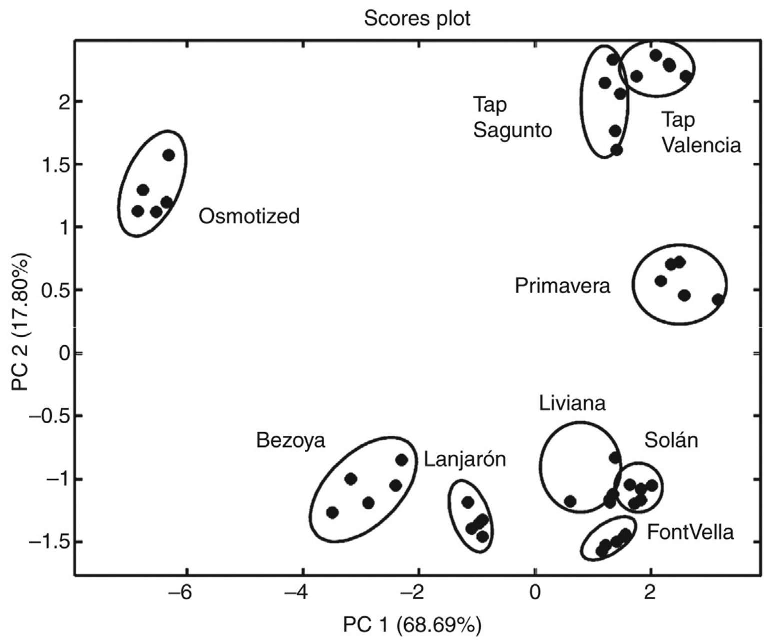

**FIGURE 25.6** **Principal component analysis (PCA) score plot for different waters.** Data is shown from five different trials. The PC axes are calculated to lie along lines of diminishing levels of variance in the data set. *(Reproduced from Martinez-Manez et al., 2005b, with permission from Elsevier.)*

increase with an increase of the number of samples for the training. However, the amount of memory use is different for the three networks when increasing the number of training samples. The memory use for MLFF and LDA does not increase with the increasing number of training samples, but MLFF requires many more training samples. On the contrary, requiring the most memory use, fuzzy ARTMAP showed an increase of the memory use with increasing of the number of training samples. Therefore, LDA was identified as the best pattern recognition algorithm and was implemented on the microcontroller.

## 25.5 CONCLUSIONS AND FUTURE ASPECTS

The continued contamination threats and an increased need for quality monitoring will guide the progress of e-tongue measurement technologies applied for drinking water analysis. The ever-increasing data handling and analysis capabilities are expected to lead to the creation of global systems for remote monitoring of drinking water.

Being an important drinking water resource, surface waters require a high level of control due to the possible contaminations by xenobiotic chemicals and pathogenic microbes originating from various anthropogenic sources. To take efficient measures against surface water pollution, relevant source assessment is required. The concept of anthropogenic burden markers, that is, environmentally stable and source-specific substances quantitatively representing the contamination, has appeared recently for the purpose of finding contamination pathways (Buerge et al., 2009; Takada and Eganhouse, 1998). Chemical markers possess a higher source specificity and higher stability compared to the bacterial markers. Revealing a quantitative correlation between concentration in natural raw water and anthropogenic burden by domestic wastewater, caffeine is considered as a potential chemical marker for domestic wastewater contamination (Seiler et al., 1999). Up to date, the most promising markers are the xenobiotics with high stability toward biodegradation (Nakada et al., 2008). Antibiotics might be used as nonanthropogenic pollution markers in surface waters, but are also important for wastewater monitoring. An ideal marker for the detection of domestic wastewater in natural waters (groundwater) is the sulfonamide-family sweetener acesulfame, which has been detected in several tap water samples from Switzerland (Buerge et al., 2009). The highest concentrations of acesulfame in groundwater were observed in areas with significant infiltration of river water receiving considerable discharges from wastewater treatment plants. The appearance of diesel and the petrochemical hydrocarbons in the raw water inlet of waterworks, as a result of leakages from boats and spillage, can cause an odor appearance in tap water (Hedström et al., 2009). Here too, the unique operational properties of e-tongue instruments in combination with possibilities of detection and classification of anthropogenic markers are expected to be highly valuable in the future for precise drinking water quality monitoring.

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## Chapter 26

# Electronic Tongues for the Organoleptic Characterization of Wines

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## 26.1 INTRODUCTION

Wine is an alcoholic beverage, consisting of several hundreds of components in different ranges of concentrations. The quality control is usually carried out by trained experts that evaluate the organoleptic properties of wines (flavor, taste, and color). Such evaluation is accomplished along the elaboration process as well as in the final product. Wines are also characterized by traditional chemical techniques that provide information about specific parameters. From the analytical point of view, the chemical analysis of wines is a challenging task due to the complexity of the mixture. Moreover, chemical differences between wines with distinct organoleptic characteristics often rely in minute differences in the concentration of certain compounds. The situation becomes even more complex because the synergy between groups of compounds often has a stronger influence in the organoleptic characteristics than individual compounds (Jackson, 2014).

To obtain a complete picture of the composition of wines, a combination of analytical techniques is needed. Usually, the number of parameters to be analyzed is simplified as a function of the aim. Therefore, only a few parameters are periodically checked. Among them, the most common are: soluble solids, reducing sugars, alcoholic degree, pH, total and volatile acidity, sulfur dioxide, color, polyphenol index, and organic acids. Deeper analysis carried out by chromatography or spectroscopy can give information about other compounds (carbohydrates, acids, alcohols, phenolic compounds, inorganic constituents, and other minor flavor components) (Smyth and Cozzoino, 2013). The analysis of wines requires the development of technologies able to detect simultaneously a large spectrum of compounds providing global information about the sample instead of information about specific components.

In recent years, electronic tongues (e-tongues) have been developed. They are based on the combination of nonspecific chemical sensors with partial sensitivity (cross-sensitivity) to different components. The response of the sensor array toward a certain sample is a collection of signals that can be related to certain features or qualities of the sample using the appropriate software. E-tongues are normally used to give qualitative answers about the sample studied and only in some cases to predict the concentration of individual species (Zeravik et al., 2009; Riul et al., 2010; Baldwin et al., 2011; Tahara and Toko, 2013; Lvova et al., 2013; Sliwinska et al., 2014).

This chapter describes the state of the art of the e-tongues showing the different types of sensors used in the analysis of wines. Current applications in oenology are presented and the possible future applications in this field are discussed.

## 26.2 PRINCIPLES OF E-TONGUES

According to the IUPAC, an e-tongue is a multisensor system, which consists of a number of low-selective sensors and cross-sensitivity to different species in solution, and an appropriate method of pattern recognition and/or multivariate calibration for data processing (Vlasov et al., 2005).

### 26.2.1 Multisensor Systems

The heart of any e-tongue is the array of sensors. Much effort has gone into developing new sensors with improved characteristics. Sensors used in e-tongues can use several measurement principles including mass, optical, or electrochemical transduction. Electrochemical sensors (including potentiometric, amperometric, voltammetric, and impedimetric sensors) are the most widely used sensing units in e-tongues because of their specificity, high sensitivity, short response time, and simple operation (Del Valle, 2010; Kimmel et al., 2012). In potentiometric sensors, a working electrode covered with a membrane is immersed in the studied solution. A potential is then created at the membrane/solution interphase, which depends on the nature of the electrode material and on the composition of

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the solution. Potentiometric sensors can be prepared from different materials, membranes, and techniques (Ciosek and Wróblewski, 2011). Silicon planar technology can be used to fabricate miniaturized devices using different transducer structures: ion selective field effect transistors (ISFETS), light addressable potentiometric sensor (LAPS), or micro ion selective electrodes (μISE) (Bratov et al., 2010). Arrays of potentiometric sensors with different selectivity and sensitivity have been successfully used to analyze wines (Paolesse et al., 2008; Zeravik et al., 2009).

Amperometric and voltammetric sensor arrays have also attracted considerable attention for the analysis of wines (Parra et al., 2004; Scampicchio et al., 2008; Winquist, 2008). In such sensors, a bias voltage is applied, while the current is measured. Voltammograms show peaks associated with the oxidation and reduction of the molecules present in the solution and their intensity is proportional to the concentration. The system is versatile because different electrode materials and excitation functions can be applied (eg, cyclic voltammetry, pulse voltammetry, or square wave voltammetry).

The modification of the electrode surface with electroactive and electrocatalytic materials (eg, phthalocyanines, perylenes, conducting polymers, or nanoparticles) gives the electrodes improved selectivity (Parra et al., 2004, 2006a; Rodriguez-Mendez et al., 2008). Voltammograms show redox peaks associated with both—the electrode and the solution. Simultaneously, the interactions between the electrode and the solution improve extraordinarily the selectivity of the electrodes. Such interactions include among others: (1) the oxidant or reducing character of the solution that can modify the oxidation potential of the electrode material; (2) the electrocatalytic activity of the electrode material that can facilitate the oxidation of the compounds solved in the test solution; (3) the acid or basic character of the solution can protonate/deprotonate the electrode; (4) the nature and concentration of ions present in the solution that diffuse inside the sensing layer to maintain the electroneutrality.

Impedance spectroscopy has also been used as a transduction method to analyze wines. Electrodes modified with various organic materials (including conducting polymers, perylenes, phthalocyanines, or carbon nanotubes) have demonstrated their capability to detect molecules present in wines (Volpati et al., 2012).

Finally, multitransduction systems are increasingly more popular, since they combine different classes of sensors that provide complementary information (Baldacci et al., 1998; Di Natale et al., 2000; Rodríguez-Méndez et al., 2004; Gutiérrez et al., 2010; Lvova et al., 2015).

More recently, bioelectrochemical sensors have been successfully introduced in sensor arrays. These systems have been called bioelectronic tongues and have attracted an increasing interest because they combine the advantages of classical arrays, which provide global information about the sample, with the typical specificity of biosensors (Zeravik et al., 2009; Toko, 2013). The enzymes incorporated in arrays dedicated to the analysis of wines include phenoloxidases specific for the detection of phenols (eg, tyrosinase, laccase, or peroxidase; Cetó et al., 2014a) and enzymes specific for the detection of sugars (glucose oxidase or fructose dehydrogenase; Gutierrez-Capitán et al., 2014; Medina-Plaza et al., 2014a ).

Nanotechnology can help significantly to improve the sensitivity and the response time of sensors and biosensors (Medina-Plaza et al., 2014b). On one hand, nanotechnology provides new nanomaterials (nanoparticles, nanocarbons, etc.) with improved electrocatalytic properties (Cetó et al., 2014b; Orozco et al., 2009). On the other hand, traditional sensing materials can be deposited as nanostructured films, using self-assembling monolayer (SAM), layer by layer (LbL), or the Langmuir–Blodgett (LB) techniques (Arrieta et al., 2003; Riul et al., 2004). Such films show enhanced surface-to-volume ratios that increase the sensitivity of the sensors.

LbL, SAM, or the LB techniques are of special interest in the field of biosensors, since using these methods, enzymes can be immobilized in lipidic layers with structures similar to those of the biological membranes. This biomimetic environment can help to preserve the functionality of the enzyme (Apetrei et al., 2011; Medina-Plaza et al., 2014b).

The key step in e-tongues is to select the most suitable sensors for each application. Secondly, the sensors combined in an array configuration must show cross-selectivity (ie, be sensitive toward different species) or at least must show cross-sensitivity (ie, react toward the same compounds but with different intensity). The combination of responses can be related to certain features or characteristics of the samples by means of pattern recognition routines.

### 26.2.2 Multivariate Data Treatment

The pattern recognition techniques consist of four sequential stages: signal preprocessing, dimensionality reduction, prediction, and validation. The signal preprocessing prepares the feature vector for future processing. It includes compensation for sensor drift, scaling of the data, and extracting representative parameters. The intrinsic complexity, richness, and cross-selectivity of the signals generated by voltammetric sensor arrays are an advantage because the data set contains meaningful information about the sample. At the same time, this complexity can result in difficulty of processing the data. One solution is to simplify the high dimensionality and to employ a feature extraction stage such as the wavelet transformation (WT) (Winquist, 2008), the kernel method (Parra et al., 2004), or genetic algorithms (Prieto et al., 2013).

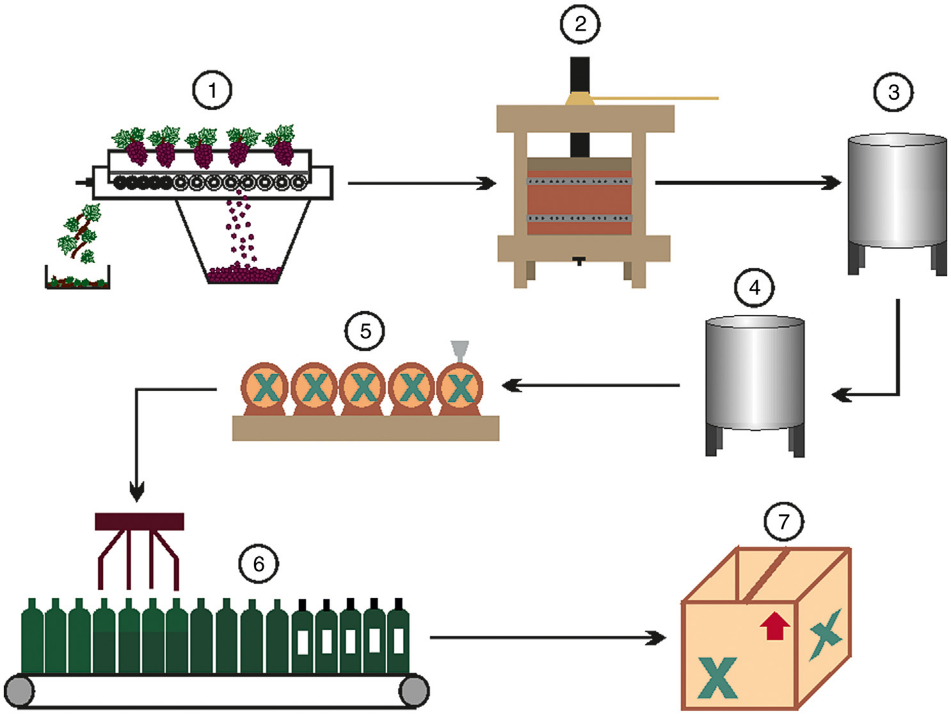

FIGURE 26.1 **Scheme of the elaboration process of wines and steps where e-tongues have been used.**

A dimensionality reduction stage projects this initial feature onto a lower dimensional space. This is usually done using a nonsupervised technique such as principal component analysis (PCA). Using PCA, it is possible to discriminate between samples with different characteristics.

The resulting low dimensional feature vector is the one used to solve a given prediction problem, typically classification, regression, or clustering. Classification tasks address the problem of identifying an unknown sample and to assign it to a certain set of previously learned categorized samples. Typical classification models used in e-tongues are linear discriminant analysis (LDA), soft independent modeling of class analogy (SIMCA), support vector machines (SVM), or artificial neural networks (ANN). In regression tasks, the goal is to establish a predictive model from a set of independent variables (eg, sensor responses) to a second set of variables that are the properties of the sample analyzed (eg, concentration, quality). They are usually carried out using partial least squares (PLS) regression models (Kirsanov et al., 2012). Finally, in a clustering task, the goal is to learn the structural relationships between different samples.

## 26.3 E-TONGUES DEDICATED TO THE QUALITY CONTROL OF WINES

Each wine has a different chemical composition that depends on the variety and maturity of grape, on the extraction of different components into the grape juice, and also, on the subsequent reactions occurring during the vinification, the postfermentation treatments, and during wine aging (Fig. 26.1). The chemical composition has a direct effect on the organoleptic properties of wines.

In spite of the fact that e-tongues do not measure mouth feels or human perceptions, as they respond to chemical compounds, they have been used to analyze or control different steps of the wine production, from the evaluation of the quality of grapes to the analysis of the bottled product.

### 26.3.1 Analysis of the Quality of Grapes (Step 1) and Pressing (Step 2)

The maturity and quality of grapes is typically established on the basis of their sugar content. The phenol content also changes with ripening. E-tongues using ISFETS (Moreno-Codinachs et al., 2008) or voltammetric biosensors (Medina-Plaza et al., 2014a) can discriminate grapes according to their variety and vintage. They can also be used to evaluate the sugar and the phenolic content of mature grapes.

During maceration, the contact between must and skins increases the concentration of phenols in wine. The extraction of phenols can be improved using grape-pressing techniques such as Flash Release. A voltammetric e-tongue was able to detect the increase in the phenol concentration after use of Flash Release and microoxygenation (Prieto et al., 2011).

## 26.3.2 Fermentation (Steps 3 and 4)

Wine is the product resulting from the fermentation of fresh grape juice or must. In the fermentation of white wines, bacteria present in the skin of grapes transform sugars in ethanol. Vinification of red wines requires two sequential fermentations. The alcoholic fermentation (transformation of glucose in ethanol) is followed by the malolactic fermentation where malic acid is transformed in lactic acid. Fermentations require a careful control of the operation conditions since small deviations (ie, in the temperature) can result in unwanted organoleptic characteristics (Peris and Escuder-Gilabert, 2013). Parameters usually measured to control the alcoholic fermentation are temperature and density. In malolactic fermentation, malic acid concentration must be monitored periodically. Because fermentation is a turbulent process, it is difficult to monitor it using an e-tongue. In spite of the difficulties, it has been demonstrated that e-tongues can monitor fermentations in several food products (ie, dairy products). However, only a few reports have been published in the field of wines. For instance, a potentiometric e-tongue combined with an electronic nose and optical measurements has been used to follow the kinetics of the fermentation process of eight musts with acceptable correlations with sugar consumption and alcohol production (Buratti et al., 2011).

## 26.3.3 Aging in Barrels (Step 5)

Traditionally, red wines are aged in oak barrels about 1 to 2 years before bottling. The organoleptic characteristics of wines are influenced by the retention and release of volatile compounds by lees during the aging process. The length of time that a wine is kept in a barrel improves the quality of wine. The porosity of the oak facilitates diffusion of minute amounts of oxygen into the barrel. The geographic origin of the oak and the degree of toast of the wood can also induce different flavors. The oxidative aging in barrels is followed by an aging in bottles that occurs in a reductive environment and that improves the organoleptic characteristics.

An e-tongue formed by potentiometric sensors was used to classify Port wines of different ages (from 2 to 70 years) (Rudnitskaya et al., 2007). The e-tongue predicted the Port wine age with accuracy similar to that obtained using chemical analysis data. In a following work, the potentiometric e-tongue was used to analyze Madeira wines produced from different varieties of grape and aging duration. It was demonstrated that the effect of age was more significant for the e-tongue than the variety of grape (Rudnitskaya et al., 2010).

A voltammetric e-tongue based on chemically modified electrodes has been successfully applied to monitor the aging of red wines and to discriminate wines aged in oak barrels of different origin (French, Lithuanian, or American) and toasting level. The array of voltammetric sensors has been able to follow the changes experienced by red wines aged in oak barrels after 3 and 6 months of aging (Parra et al., 2006b).

Aging in oak barrels requires long times with a high cost. For this reason, recently, innovative aging methods have been developed. One of these techniques consists in accelerating the aging of wines by soaking pieces of wood of different sizes (chips or staves) in micro-oxygenated stainless-steel tanks. A voltammetric e-tongue has been used to detect the use of such practices. In the early stages of aging, the e-tongue was able to discriminate between wines aged with traditional and alternative methods due to the faster rate of aging caused by chips or staves. The effect of the size and of the type of the pieces of wood could also be evaluated. After 5 months of aging, the use of alternative methods could be no longer detected. However, when the aging continued in a reducing atmosphere (bottled wines), the e-tongue could discriminate wines aged in oak barrels from those previously treated with oak chips (Apetrei et al., 2007; Gay et al., 2010).

## 26.3.4 Bottling (Step 6)

The stoppers used as closures are traditionally made of natural cork. They are ideal to seal liquid and gas while allowing diffusion of minute amounts of oxygen from the outside. Natural stoppers are associated with high-quality wines. The wine industry has now available polymeric stoppers with controlled porosity able to deliver known and reproducible amounts of oxygen into bottles through the closures (controlled oxygen transfer rate: OTR). Recently, the effect of this nanooxygenation has been successfully analyzed using an e-tongue (Prieto et al., 2011). It was demonstrated that the e-tongue is more sensitive to the different OTRs than the electronic nose (Rodriguez-Mendez et al., 2014).

## 26.3.5 Evaluation of the Final Product (Step 7)

The final step in aging wines occurs in the reductive environment provided by bottles. E-tongues have been able to monitor the aging in bottle and to evaluate the changes in the organoleptic properties that occur naturally (improving the quality of wines), but can also detect unwanted changes due, for instance, to inappropriate storage or cork damage.

### *26.3.5.1 Evaluation of the Organoleptic Properties Produced by the Variety of Grape, Vintage, Appellation, etc*

The grape variety from which a wine is produced determines the flavor and organoleptic characteristics of the final product. Moreover, each appellation establishes the grape varieties that can be grown and other quality parameters, as well as the oenological manipulations permitted or coupages accepted. In addition, each winemaker has his own method to elaborate wines (eg, crushing, use of yeast, temperature control, etc.). There are thousands of grape varieties and oenological techniques producing a large number of different wine styles having its own distinct flavors and characteristics. On the top of this, the weather conditions influence the quality of each vintage. Finally, as the aging continues in the bottle, the chemical composition and organoleptic properties change with time, improving the bouquet. Too long or inappropriate storage conditions can spoil the wine.

The number of variables is so high that the training of an e-tongue requires the use of experimental wines purposely elaborated for this particular use.

Arrays of potentiometric chemical sensors have been extensively used to analyze wines. For instance, they were used to discriminate Italian wines (20 samples of Barbera d'Asti and 36 samples of Gutturnio wine) from the same appellation and vintage but from different vineyards (Legin et al., 1999, 2003). Arrays of all-solid-state potentiometric sensors covered with PVC membranes containing porphyrins discriminated Italian white wines of Verdicchio appellation (Verrelli et al., 2007a). A set of 14 Madeira wines produced from 4 varieties (Bual, Malvasia, Verdelho, and Tinta Negra Mole) that were 3, 6, 10, and 17 years old was analyzed using an array of 26 potentiometric sensors with plasticized PVC and chalcogenide glass membranes. It was found that effects of age, grape variety, and their interaction were significant for the HPLC data set and only the effect of age was significant for the e-tongue data (Rudnitskaya et al., 2010).

An ISFETs-based array was suitable to distinguish grape types and vintage of wine samples (Artigas et al., 2003; Moreno-Codinachs et al., 2008). Multiparametric systems combining different types of sensors (ISFETs, conductivity, redox potential, and amperometric) are also a good alternative capable of characterizing and classifying monovarietal white wines according to the grape variety and geographical origin (Gutiérrez et al., 2010).

Amperometric detection combined with an electronic nose could classify Italian wines having different denominations of origin and produced in enclosed geographical areas (Buratti et al., 2007).Voltammetric measurements using electrodes modified with phthalocyanines have been widely used to discriminate wines of different Spanish regions (Rioja, Rueda, Ribera de Duero) and aging (Parra et al., 2004; Rodríguez-Méndez et al., 2004). The array was extended to 12 sensors modified with 3 families of electroactive materials (phthalocyanines, conducting polymers and perylenes) and used to analyze monovarietal wines from four Spanish regions (Ribera de Duero, Toro, Bierzo, and Cigales). The same samples were elaborated in vintages ranging from 1998 to 2000. The system easily classified wines according to the variety of grape, whereas differences from vintage to vintage were less marked (Parra et al., 2006c; Rodriguez-Mendez et al., 2008). Using also voltammetric techniques, an array of electrodes modified with phthalocyanines or nanoparticles has been applied to the classification of cava wines based on their different aging times (Cetó et al., 2015).

Impedimetric gold interdigitated sensors modified with conducting polymers/lipids and chitosan can also correctly distinguish red wines, according to the vintage, vineyard, brand, and storage conditions (Riul et al., 2004).

Introducing biosensors in the array can improve the performance of the e-tongues. However, combining biosensors in a multisensor configuration is difficult since each enzyme has its optimal working conditions, requiring particular immobilization conditions and appropriate electron mediators. In spite of the difficulties, there is an increasing interest toward the application of bioelectronic tongues to the analysis of wines. For instance, graphite–epoxy voltammetric biosensors containing tyrosinase or laccase have demonstrated their utility to analyze cava wines (Cetó et al., 2014b). Carbon paste electrodes modified with tyrosinase, laccase, and glucose oxidase, using phthalocyanines as electron mediators, have been able to discriminate grape juices prepared from different varieties of grapes (Medina-Plaza et al., 2014a).

### 26.3.5.2 Monitoring Spoilage

Potentiometric e-tongues have been used to monitor the spoilage of wines that was also followed by the titrable (total) acidity (Gil-Sanchez et al., 2011). Similarly, an e-tongue composed of "all-solid-state" potentiometric sensors was able to monitor the levels of acetic acid in white wines, indicating the wine spoilage process (Verrelli et al., 2007b).

### 26.3.5.3 Detection of Frauds and Adulterations

Wine producers must follow the practices specified by their appellations and by their national and international regulations. Wine adulteration can be committed through dilution with water, addition of alcohol or other substances, blending with, or replacement by, wine of a lesser quality or elaborated from varieties of grapes from a different region, and by using forbidden aging methods. As demonstrated in the previous paragraphs, e-tongues can be used to discriminate wines elaborated using different grapes and techniques. This ability can be applied in the control of frauds, complementing the traditional analytical techniques.

In fact, a voltammetric e-tongue could detect model fraudulent red wines obtained by adding a range of forbidden adulterants (ethanol, tartaric acid, tannic acid, $SO_2$, acetic acid, sucrose, and ethanol) (Parra et al., 2006c). Similarly, using miniaturized potentiometric sensors, wine defects caused by $H_2S$, $SO_2$, and acetic acid were identified in artificial wines (Verrelli et al., 2007b).

## 26.4 ASSESSMENT OF CHEMICAL PARAMETERS

E-tongues are holistic systems that provide global information about a sample instead of information of particular components. For this reason, most papers devoted to the evaluation of wines using e-tongues deal only with classification tasks using PCA. However, the signals provided by the sensor array are correlated with the chemical composition of the sample. The analysis of the data matrix with adequate chemometric tools can provide information of particular parameters (Kirsanov et al., 2012; Oliveri et al., 2010). Total polyphenols, sugar content, total and volatile acidity, pH, etc. can be predicted from the e-tongue responses. PLS regression is the most widely used method to obtain calibration models for numerical predictions of various quality parameters. Although good correlations can be found using different types of e-tongues, when interpreting the results, it has to be taken into account that the number of samples used to establish the mathematical models is usually low. This is due to the difficulty of having available large sets of well-controlled and characterized wines, which must be purposely prepared for these studies. The main advantage is that, once calibrated, e-tongues can predict several chemical parameters simultaneously.

Potentiometric, ISFETs, amperometric, voltammetric, and impedimetric sensors have been used for this task. Each type of transduction has its own characteristics and advantages.

Potentiometric sensors are particularly suitable to quantify the presence of ions (eg, acidity, calcium, or heavy metals), although the membrane potential can be also produced by the presence of other components. For instance, using a potentiometric e-tongue, total and volatile acidity, pH, ethanol content, tartaric acid, sulfur dioxide, total polyphenols, and glycerol have been quantified in Italian wines with precision within 12% (Legin et al., 2003).

Two arrays of metalloporphyrins-based gas and liquid sensors were able to quantify the following parameters in a red wine: sugar, acidity, pH, tartaric, malic and lactic acids, polyphenols, antocyans, and ions (Ca, Mg, and K). Results demonstrate the capability of such systems to be trained according to the behavior of a practical panel of tasters (Di Natale et al., 2000, 2004).

Porphyrin-based potentiometric sensors were used to detect alcohols in beverages (Lvova et al., 2006). This system was also chosen for quantitative analysis of dry white wines of "Verdicchio" appellation. A satisfactory correlation between results of wine analysis performed by certified methods and e-tongue response has been obtained for $SO_2$, L-malic acid, and total phenols index (Verrelli et al., 2007a). A miniaturized version of this device identified wine defects caused by $H_2S$, $SO_2$, and acetic acid as markers in artificial wines (Verrelli et al., 2007b).

An e-tongue formed by potentiometric sensors with plasticized PVC and glass membranes was capable of detecting the following components in Madeira wines (mean relative error in cross-validation is shown in the parentheses): tartaric (8%), citric (5%), formic (12%), protocatehuic (5%), vanillic (18%), and sinapic (14%) acids, catechin (6%), vanillin (12%), and trans-resveratrol (5%) (Rudnitskaya et al., 2010). Similarly, an e-tongue based on a potentiometric platform was used to analyze 36 white wines from New Zealand, to assess the contents of free and total sulfur dioxide, total acidity, ethanol, pH, and some phenolics (Kirsanov et al., 2012). A multiparametric analyzer based on ISFET sensors was used to measure pH, calcium, and potassium, necessary to control the tartaric stabilization of wines (Artigas et al., 2003). The combination of electrochemical microsensors and a colorimetric optofluidic system has been able to quantify several sample parameters of interest in wine quality control (Gutiérrez et al., 2010; Gutierrez-Capitán et al., 2014).

Voltammetric and amperometric sensors are sensitive to the electroactive species present in the studied solution. This is particularly interesting for the analysis of wines because they contain a large number of compounds with redox activity. They include polyphenols, which are responsible of the antioxidant properties of wines, sugars (which have a reducing character), and other compounds such as sulfites, which are added to stabilize wines. For instance, an e-tongue based metallic electrodes with a pulse voltammetry has been used to detect bisulfites (Labrador et al., 2009). When the sensing units are modified with electrocatalytic materials, the intensity of the signals increases, improving the sensitivity in this way (Arrieta et al., 2003).

Several papers have been published in which Spanish wines have been characterized by chemical analysis and using an e-tongue based on voltammetric electrodes modified with electrocatalytic materials. Correlations with 24 chemical parameters were established. The e-tongue was demonstrated to be particularly useful to estimate the polyphenolic content of wines (measured as the total polyhenol index), with specific phenolic compounds (as measured by chromatography) with color parameters (measured by spectroscopy) and acidity (pH or total acidity) (Apetrei et al., 2007; Parra et al., 2004; Prieto et al., 2011; Rodriguez-Mendez et al., 2014).

Other electrocatalytic modifiers such as ferrocene or nanoparticles have also been successfully incorporated in voltammetric sensors, and have been employed to estimate the antioxidant capacity of wines (Cetó et al., 2012, 2014a; Del Valle, 2010).

The accuracy of the predictions can be improved by including biosensors in the array. The specificity enzyme-substrate facilitates the assessment of parameters such as phenols (when including phenol oxidases), or sugars (when including glucose oxidase or fructose dehydrogenase) (Gutierrez-Capitán et al., 2014). Several immobilization methods or electron mediators have been tested, but bioelectronic tongues are at the beginning of their development in the field of wines.

## 26.5 PREDICTION OF SCORES GIVEN BY A PANEL OF EXPERTS

One of the final goals in e-tongues is to find correlations between the electronic signals and the scores given by a panel of experts. This is a complex task, due to the complexity of human perceptions. The human gustatory sense detects substances with taste, and also perceives mouthfeel (astringency, heat, viscosity, etc.) that contributes to the perception as well. Unlike the human systems, e-tongues base their evaluations on the analysis of chemical compounds whatever they are taste or tasteless species. Thus, it is mandatory to keep in mind that an absolute taste description is not possible from an e-tongue point of view.

In the field of wines, it is particularly difficult to establish correlations between the scores given by a panel and the output of e-tongues. First, wines are extremely complex mixtures; second, in many cases, the intensity of a perception is not correlated with the concentration of a certain compound. In addition, the scores given by a panel of experts evaluate how pleasant is the flavor, but the perception is not linearly related to a compound or a family of compounds. For instance, high scores in sourness are given to wines with an intermediate acid concentration (not high, not low). In other words, linear mathematical models are not the most appropriate models. In spite of the difficulties, several works have reported good correlations with sensory scores using PLS regression and potentiometric (Di Natale et al., 2004; Kirsanov et al., 2012; Legin et al., 2003), amperometric (Buratti et al., 2007), or voltammetric sensors (Cetó et al., 2015; Gay et al., 2010). Further research is needed in this field.

## 26.6 CONCLUSIONS AND FUTURE TRENDS

E-tongues based on different sensing principles (potentiometry, voltammetry, etc.) can be a valuable tool to be used together with classical quality control techniques. During production, they could be used to carry out routine analysis, helping the oenologists to make key decisions regarding harvesting, crushing, fermentation, type of barrel to be used for the aging, among many other decisions. They could be used to control the quality of the end product but also by official organisms to detect frauds and illegal practices.

The absolute taste description is not possible from an e-tongue point of view, but they provide an objective response that does not depend on physiological conditions or personal preferences; they do not show fatigue (as human taste testers do). So, e-tongues are valuable tools to complement the information obtained by chemical or sensorial analysis.

Future strategies will include the design of arrays formed by new materials with improved selectivity, sensitivity, and catalytic properties. Advances in bioelectronic tongues are also expected, incorporating new enzymes or combinations of more than one active molecule and developing new immobilization methods.

The most important advances in the performance of electrochemical sensors and biosensors will be linked to nanotechnology. Efforts have to be made to develop new nanostructured sensors while lowering the fabrication costs.

Efforts must also be made to introduce these instruments in cellars and in the list of recommended analytical tools established by national and international commissions.

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## Chapter 27

# Olive Oil and Combined Electronic Nose and Tongue

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## 27.1 INTRODUCTION

Olive oil is an extract from olive fruits. It is obtained by triturating the olives and pressing of the paste produced. The juice obtained from the olive paste is a mixture of water and oil. This juice is unstable and when the two phases are separated, the oily phase results in virgin olive oil (VOO). When VOO is extracted from ripened and healthy olive fruits (*Olea europaea* L.) and suitably processed and stored, it shows a characteristic flavor that is highly appreciated by consumers (Gunstone, 2002).

Extra virgin olive oil (EVOO) is a natural oil, rich in vitamin E (a robust antioxidant), pigments (responsible with greenish or yellowish colors, chlorophylls and carotenoids), and polyphenols, which give stability, aroma, and taste of olive oils (Aparicio and Harwood, 2013). Pigments and polyphenols are healthy, beneficial compounds that are excellent protectors of cardiovascular vessels in the human body (Dai and Mumper, 2010). In conclusion, the olive oil is much more than one fat type because it is a complex of useful substances for human health.

### 27.1.1 Compounds Related to the Taste of Olive Oils

Olive oil contains a high proportion of triglycerides together with small amounts of free fatty acids, monoglycerides, diglycerides, hydrocarbons, sterols, and phenolic compounds. Minor compounds are very important for the stability, aroma, and taste of olive oil. In addition, quantitative analysis of these compounds is useful for authentication and detection of fraud and adulteration (Boskou, 2006).

Sensory assessment was introduced for the standardization of the VOO market in 1970. In this regard, the acceptance of extra virgin olive oil by consumers has been often related to the flavor, which includes taste and odor. The main VOO taste attributes are bitterness, pungency, and astringency. These attributes are concerned with the presence of secondary metabolites from the class of phenolic compounds, which originate from those existing in the olive fruit. The composition related to the phenolic compounds from olive fruits are quantitatively and qualitatively different depending on its cultivar, farming practices, water availability, temperature, etc. (Monteleone and Langstaff, 2014).

Phenolic compounds, as broad term, refer to a large number of compounds (more than 8000), widely dispersed throughout the plants. They are characterized by having at least one phenolic moiety, conjugated or not (Aparicio and Harwood, 2013).

Most of phenolic compounds are produced in plants as secondary metabolites via the shikimic acid pathway. The biosynthesis of phenolic compounds is catalyzed by phenylalanine ammonia lyase and the substrate is phenylalanine, an aromatic amino acid.

Phenolic compounds are categorized into nine groups (Dey and Harborne, 1989):

- phenols, phenolic acids, phenylacetic acids
- lignans
- lignins
- flavonoids
- cinnamic acids, coumarins, isocoumarins, and chromones
- tannins
- benzophenones, xanthones, and stilbenes
- quinones
- betacyanins.

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Other phenolic compounds, based on the number and arrangement of their carbon atoms, are found in two categories (Crozier et al., 2006):

- flavonoids (flavonols, flavones, flavan-3-ols, anthocyanidins, flavanones, isoflavones and others)
- nonflavonoids (phenolic acids, hydroxycinnamates, stilbenes, and others)

The polyphenol content differs from VOO to VOO. Wide ranges were reported in the literature (50–1000 mg/kg) but the values are usually between 100 and 300 mg/kg (Apetrei and Apetrei, 2013). The cultivar, the system of extraction, and the conditions of olive oil processing are critical factors for the content of polyphenols. Polyphenolic compounds are important for the flavor (taste and aroma) as well as the stability of olive oil. When polyphenolic content exceeds 300 mg/kg, the VOO may have a bitter taste (Therios, 2009).

Phenolic compounds principally found in VOO are presented in Table 27.1.

Several phenolic acids such as vanillic, ferulic, syringic, cinnamic, protocatechuic, *p*-hydroxybenzoic, caffeic, *p*- and *o*-coumaric, and gallic acid were identified and quantified in VOO. In all cases, the quantities are lower than 1 mg/kg of VOO. Phenolic acids are associated with the color and the organoleptic attributes (flavor and astringency) of VOO (Bendini et al., 2007).

A direct relationship between polyphenols and olive oil pungency has been assessed. For instance, ligstroside aglycon is related to the burning sensation found in many VOOs. Some phenolic compounds are responsible for the tasting perception of bitterness. The intensity of bitterness is related to the olive fruit varieties and the ripening stage. Polyphenols with a bitter taste are present in higher quantities in VOOs obtained from unripe fruits (Andrewes et al., 2003).

Among the many phenolic compounds, the oleosides (secoiridoides) are specificly for the *Oleaceae* family. Within *Olea europea* L. fruits, the main phenolic compounds are oleuropein, demethyl oleuropein (the acid derivative of

**TABLE 27.1 Phenolic Compounds in VOO**

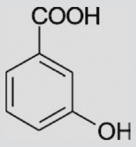
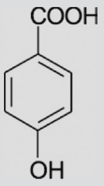
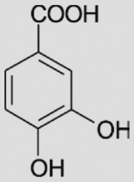
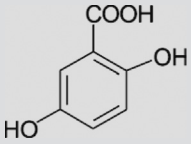
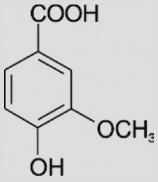
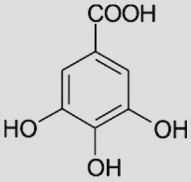
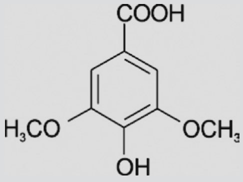
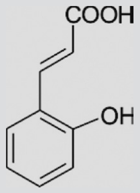
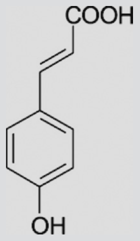
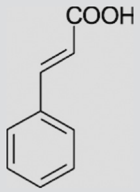
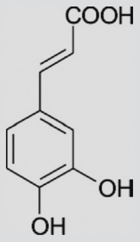
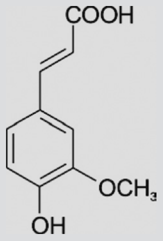
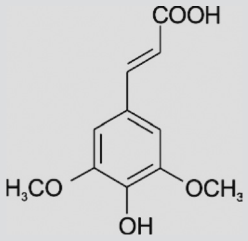
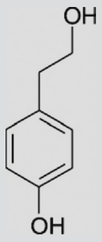
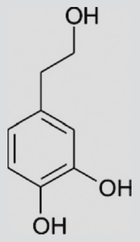
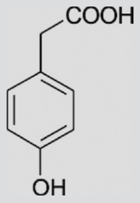
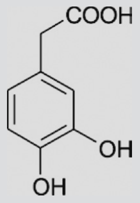
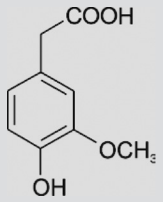
| Class | Compound | Chemical Structure |
|---|---|---|
| Benzoic acids and derivatives |  <br>3-Hydroxybenzoic acid |  <br>*p-* Hydroxybenzoic acid |
| |  <br>3,4-Dihydroxybenzoic acid<br>(protocatechuic acid) |  <br>2,5-Dihydroxybenzoic acid (gentisic acid) |
| |  <br>Vanillic acid |  <br>Gallic acid |
| |  <br>Syringic acid | |

**TABLE 27.1** Phenolic Compounds in VOO (*cont.*)

| Class | Compound | Chemical Structure |
|---|---|---|
| Cinnamic acids and derivatives |  *o*-Coumaric acid |  *p*-Coumaric acid |
| |  Cinnamic acid |  Caffeic acid |
| |  Ferulic acid |  Sinapinic acid |
| Phenyl ethyl alcohols |  Tyrosol [(*p*-hydroxyphenyl)ethanol] or *p*-HPEA |  Hydroxytyrosol [(3,4-dihydroxyphenyl)ethanol] or 3,4-DHPEA |
| Other phenolic acids and derivatives |  *p*-Hydroxyphenylacetic acid |  3,4-Dihydroxyphenylacetic acid |
| |  4-Hydroxy-3-methoxyphenylacetic acid | |

(*Continued*)

**TABLE 27.1** Phenolic Compounds in VOO (*cont.*)

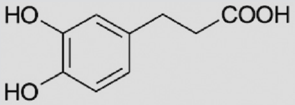
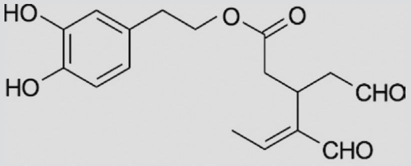
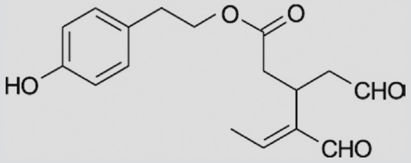
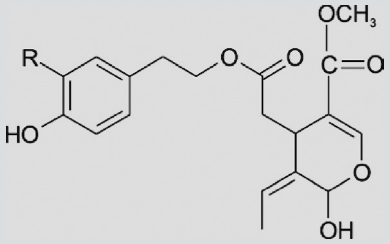
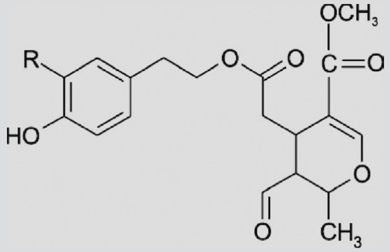
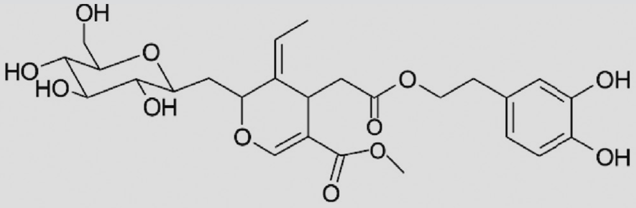
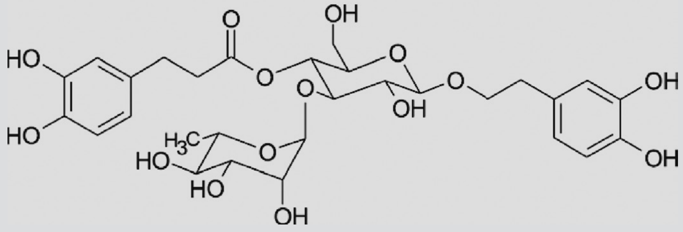
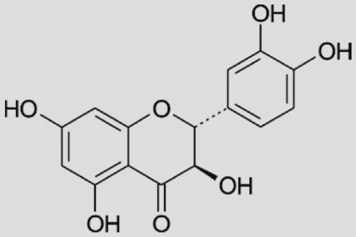
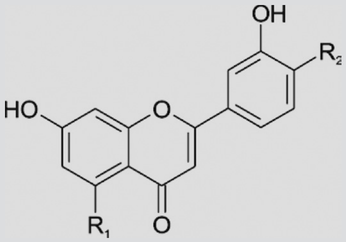
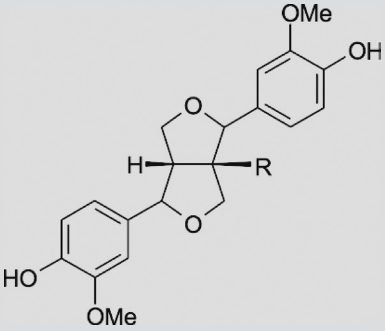
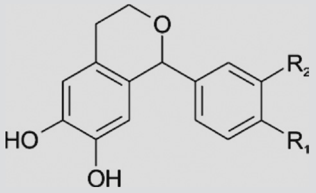
| Class | Compound | Chemical Structure |
|---|---|---|
| 3-(3,4-Dihydroxyphenyl) propanoic acid | |  |
| Dialdehydic forms of secoiridoids | | <br>Decarboxymethyl oleuropein aglycon |
| | | <br>Decarboxymethyl ligstroside aglycon |
| Secoiridoid aglycons | |  |
| | Oleuropein aglycon | Ligstroside aglycon |
| | |  |
| | Aldehydic form of oleuropein aglycon (R-OH) | Aldehydic form ligstroside aglycon (R-H) |
| Secoiridoids | | <br>Oleuropein<br><br>Verbacoside |

**TABLE 27.1** Phenolic Compounds in VOO (*cont.*)

<table>
<tr><th>Class</th><th>Compound</th><th colspan="2">Chemical Structure</th></tr>
<tr><td>Flavonols</td><td colspan="3"><br>(+)-Taxifolin</td></tr>
<tr><td rowspan="2">Flavones</td><td colspan="3"></td></tr>
<tr><td>Apigenin<br>($R_1$-OH, $R_2$-H)</td><td colspan="2">Luteolin<br>($R_1$-OH, $R_2$-OH)</td></tr>
<tr><td rowspan="2">Lignans</td><td colspan="3"></td></tr>
<tr><td>(+)-Pinoresinol<br>(R = H)</td><td>(+)-1-Acetoxypinoresinol<br>(R = $OCOCH_3$)</td><td>(+)-1-Hydroxypinoresinol<br>(R = OH)</td></tr>
<tr><td rowspan="2">Hydroxyisochromans</td><td colspan="3"></td></tr>
<tr><td>1-Phenyl-6,7-dihydroxyisochroman<br>($R_1$, $R_2$-H)</td><td colspan="2">1-(3′-Methoxy-4′-hydroxy)phenyl-6,7-dihydroxyisochroman<br>($R_1$-OH, $R_2$-$OCH_3$)</td></tr>
</table>

oleuropein), ligstroside, and verbascoside (Vazquez-Martin et al., 2012).

Oleuropein belongs to the group of compounds named secoiridoids, which are present in high quantities in the plants from the family of *Olearaceae*. The presence of oleuropein and its content are related to the ripening stage of the olive fruit. Moreover, oleuropein contributes to the bitter sensory perception (Kranz et al., 2010).

Secoiridoids of VOO in aglyconic forms originate from the corresponding glycosides found in olive fruits by hydrolysis. The process is biocatalyzed by β-glucosidases during crushing and malaxation of olives. The most abundant secoiridoids of VOO are the dialdehydic form of elenolic acid bonded to tyrosol or hydroxytyrosol and an isomer of the oleuropein aglycon (Table 27.1; Apetrei et al., 2004).

### 27.1.2 Compounds Related to the Aroma of Olive Oils

More than two hundred of compounds have been identified in the volatile fraction of VOOs. As reported in the literature, diverse volatile compounds exist in olive oil including hydrocarbons, alcohols, aldehydes, ketones, acids, esters, ethers, furan derivatives, thiophene derivatives, pyranones, thiols, and pyrazines.

From this huge number of volatile compounds, only some of them contribute to the flavor of virgin olive oils resulting in both positive and negative sensorial attributes.

The studies for determining the qualitative composition of odorants present in VOOs have demonstrated similar results. Meanwhile, the quantitative composition of aroma compounds has revealed high discrepancies among VOOs of different varieties (Guadarrama et al., 2000; Apetrei et al., 2010). VOO obtained in optimal conditions contains principally volatiles derived from linoleic and linolenic acid decomposition through the lipoxygenase pathway (Angerosa et al., 1999).

The most abundant compounds are hexanal, (E)-2-hexenal, (Z)-3-hexenal, hexan-1-ol, (Z)-3-hexen-1-ol, hexyl-acetate, and (Z)-3-hexenyl acetate. In addition to these volatiles, auto-oxidation products of fatty acids (oleic, linoleic, and linolenic), mainly aldehydes and ketones, are present in VOO. Biochemical transformations of amino acids such as phenylalanine, isoleucine, or valine, provide alcohols, and esters that can potentially contribute to the aroma of olive oil (Clodoveo et al., 2014).

As found in Kalua et al. (2007) and Lanzotti and Taglialatela-Scafati (2000), the following compounds primarily contribute to basic flavor notes:

- green: (Z)-3-hexenal, (E)-2-hexenal, (E)-3-hexen-1-ol, (E)-2-hexen-1-ol, (Z)-3-hexen-1-ol
- fruity: ethyl 2-methylbutyrate, ethyl isobutyrate, ethyl cyclohexylcarboxylate, (E)-2- hexenal, hexyl acetate, (Z)-3-hexenyl acetate, ethyl 2-methylpropanoate
- fatty: heptanal, (E)-2-nonenal, (E)-2-octenal, (Z)-3-nonenal, (E)-2-decenal
- blackcurrant: 4-methoxy-2-methyl-2-butanethiol
- grassy: hexanal, (Z)-3-hexen-1-ol
- soapy: nonanal, octanal
- deep fried: 2,4-decadienal
- sweet: phenyl acetaldehyde, hexyl acetate
- astringent-bitter: (E)-2-hexen-1-ol, (E)-2-hexenal

Numerous volatile compounds have been found in VOOs with poor quality. Some of these volatile compounds give rise to sensory defects when they are present at high levels. Acids, esters, alcohols, aldehydes, and ketones are mainly responsible for the most frequent off-flavors developed in VOO. The major contributors to the so-called *fusty* off-flavor are ethyl butanoate, propanoic, and butanoic acids. Also, 1-octen-3-ol and 1-octen-3-one are responsible for the *mustiness-humidity* VOO off-flavor, which is a characteristic for the oils obtained from fruits piled under humid conditions for several days, giving rise to the development of various kinds of fungi (Tanouti et al., 2012).

The main volatiles producing the *rancid* off-flavor are aldehydes, which are the decomposition products of linolenic, linoleic, and oleic acid hydroperoxides. Also, the chief odorants that contribute to the *winey-vinegary* off-flavor are acetic acid, 3-methylbutan-1-ol, and ethyl acetate (Morales et al., 1997).

The phenolic composition is not exclusively circumscribed to express the taste sensory descriptors since certain volatile phenolic compounds (guaiacol, 4-ethylguaiacol, 4-ethylphenol, 4-vinylguaiacol, 4-vinyl phenol) are associated with fusty and musty undesirable odors (Moran and Rajah, 1994).

## 27.2 ELECTRONIC NOSES AND ELECTRONIC TONGUES EMPLOYED IN OLIVE OILS

Many efforts were carried out to develop instrumental methods capable of determining the components responsible for the flavor of olive oils and to remove the subjectivity and other disadvantages coming from the sensory evaluation by human sensory panels. Generally, volatile compounds are determined by gas chromatography–mass spectrometry (GC–MS) (Angerosa et al., 1995; Kesen et al., 2013).

In recent years, significant efforts have been devoted to the development of the electronic nose and the electronic tongue as fast, efficient, and reliable testing methods mimicking the human sense of olfaction and of taste.

The electronic nose consists of an array of gas sensors with different selectivity, a signal collecting unit, and suitable pattern recognition software (Gardner and Bartlett, 1999). These systems are also called olfaction instruments, capable of discriminating among a wide variety of simple and complex odors.

Electronic tongues are defined as sensors arrays capable of distinguishing among very similar liquids employing the concept of global selectivity, where the response of sensors provides a fingerprint for the analyzed sample. Data acquisition systems and appropriate multivariate data analyses are also included in e-tongue technology (Vlasov et al., 2005).

The heart of any electronic nose or electronic tongue is the sensor array. Selection of sensor technology relies on many factors related to the nature of the analyte, the nature of the sample evaluation, and the condition of the analysis.

All types of sensors interact with the gas to be measured when volatile compounds flow over the sensor. In the case of the electronic tongue, the sensors are immersed in the liquid sample and the interaction take place at the interface of the solid–liquid. Due to these interactions, some physical and chemical properties of the sensitive layer are modified.

A wide variety of gas sensors are currently used for the development of electronic noses based on different materials (conducting polymers, metal oxides, metal-insulator semiconductor field effect transistors, etc.) and detection principles (piezoelectric, electrochemical, optical, calorimetric sensors, etc.). The sensors that are used to detect the molecules of chemicals are based on the measurement principles such as electrical, thermal, optical, and mass changes. In recent years, novel technologies such as mass spectrometry and ion mobility spectrometry have been introduced in the field of electronic nose as given in Table 27.2 (Patel, 2014).

The sensors used for construction of electronic tongues work based on several principles, including mass detection, optical transduction, or electrochemistry (Baldwin et al., 2011; Pearce et al., 2003; Smyth and Cozzolino, 2013).

Among the various principles of detection, electrochemical techniques (potentiometry, voltammetry) and impedance spectroscopy are the most widely used approaches. The sensitive materials used for development of the sensors are very different including phthalocyanine, conducting polymers, metals, plasticized polyvinyl chloride (PVC) containing lipid membranes, and chalcogenide glasses.

Special attention is paid to the deposition of sensitive material onto solid substrate because the sensitivity is related to morphology of surface. Sensitive materials were included on/onto sensor sensitive element by different methods such as carbon paste, thin films (produced by layer-by-layer technique, Langmuir–Blodgett technique, electrochemical deposition, etc.), and membranes (Apetrei, 2012; Apetrei and Apetrei, 2013; Cetó et al., 2014; Gutiérrez-Capitán et al., 2013; Riul et al., 2010).

The concept of the electronic tongue was extended by using biosensors capable of molecular recognition, with different applications. These systems were called bioelectronic tongues (Zeravik et al., 2009).

The electronic nose and the electronic tongue are widely used for analysis of VOOs, as well as for monitoring flavor (taste and odor) changes (Apetrei, 2012; Apetrei and Apetrei, 2013; Cetó et al., 2014; Gutiérrez-Capitán et al., 2013; Riul et al., 2010; Zeravik et al., 2009).

Use of fusion technology between the electronic nose and the electronic tongue could enhance the capability and improve the quality of information about VOO samples under study. Fig. 27.1 illustrates the block diagram of the fusion system. The integration of the electronic nose and the electronic tongue in a system is possible and the principal drawback is the different time required for measurements, with the analysis with the electronic nose being slower.

The fusion of data obtained can be followed by the data analysis. Usually, the data analysis includes reduction of dimensionality (by principal component analysis), discrimination, and classification (by discriminant analysis, partial least squares discriminant analysis, soft independent modeling of class analogy, etc.), and multivariate correlations (partial least squares correlation, partial least square regression, etc.) (Brereton, 2007).

## 27.3 FUSION OF ELECTRONIC NOSES AND ELECTRONIC TONGUES IN THE ANALYSIS OF OLIVE OILS

Smell and taste are two different sensing systems but most of the time, an interaction between both systems takes place to provide the perception of flavor. Hornung and Enns (1986) suggested that "although the smell and taste interact with one another to a great extent, we probably do not taste anything that has not been influenced the olfactory sense." In contrast, the smell sensation affects the olfactory system, but to a lesser amount. Perception of flavor is influenced

**TABLE 27.2** Principle, Magnitude, and Type of Sensors Used for Electronic Nose

| Detection Principle | Magnitude | Sensor | Sensitive Material |
|---|---|---|---|
| Conductometric | Resistance/conductance | Metal oxide gas sensor | ($SnO_2$, GaO) |
| Potentiometric | Voltage | Ion selective field-effect transistor | Catalytic metals |
| Capacitive | Capacitance/charge | Humidity sensor | Polymeric materials |
| Amperometric | Current | Electrochemical cell | Solid or liquid electrolytes |
| Calorimetric | Heat/temperature | Pellistor gas sensor | Catalyst-loaded ceramic |
| Gravimetric | Mass change (frequency shift) | Piezoelectric or surface acoustic wave (SAW) sensors | Organic or inorganic film layers |
| Optical | Pathlength/absorption | UV-Vis, infrared detector | Organic dyes |
| Resonant | Frequency | Surface plasmon | |
| Fluorescent | Intensity | Fiber optic | Fluorescent-light emission material |

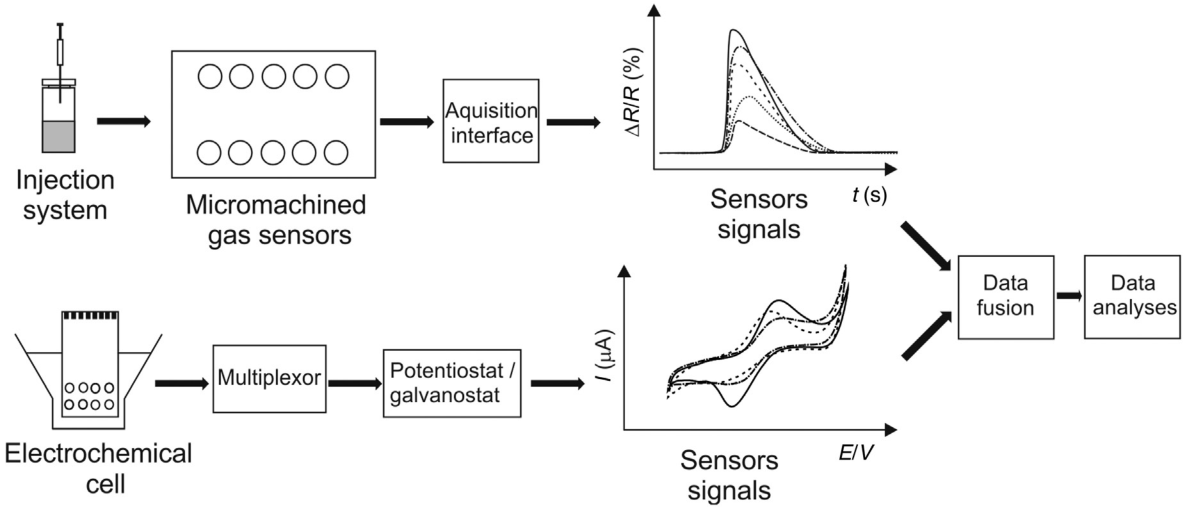


**FIGURE 27.1** The block diagram of the fusion between the electronic nose and the electronic tongue.

by all the other senses, producing an enormous information flow, which is needed to be processed.

The collection of data that comes from diverse sensors creates complementary description about the product. Integration of information resulting from different analytical tools is called data fusion, which includes processing huge multivariate signals with different origins (Ouyang et al., 2014). This idea has attracted much interest around the world to consider for olive oil authentication. Among the diverse analytical techniques applied to olive oil authentication in the last decade, fusion of electronic tongue and electronic nose as two kinds of fast, noninvasive, nondestructive, and reliable approaches has been widely used. In the literature, data fusion of numerous methods such as spectroscopy, image analysis, electronic nose, electronic tongue, chemical analysis, ultrasound, and other analytical methods for many kinds of foods and specifically for olive oil authentication have been reported (Reid et al., 2006; Ghasemi-Varnamkhasti et al., 2010; Cole et al., 2011; Casale et al., 2012; Peris and Escuder-Gilabert, 2013; Ouyang et al., 2014; Haddi et al., 2014).

Chemosensors systems such as electronic noses and electronic tongues have grown in interest and application over the last decades in parallel with the implementation of the chemometrics tools.

Recently, there have been attempts for fusion of electronic noses and electronic tongues in order to achieve the improved classifications for olive oil. An electronic nose and electronic tongue fusion system was found to noticeably enhance classification properties. It can be expected that the fusion of electronic tongue and electronic nose is useful, mainly for such measurement circumstances in which the changes are important in both the liquid and gas phases. Fusion of electronic nose and electronic tongue to olive oil analysis is the focus of this chapter; it is worth mentioning there are few reports on the fusion of electronic nose and electronic tongue for the olive oil authentication and characterization.

Di Natale et al. (2001) designed and developed a fusion data system in order to define the olive oil characteristics. They emphasized that due to the complex nature of olive oil, data fusion of different sensors would lead to better evaluation of this product.

Since olive oil has a complex nature, the use of just electronic tongue or electronic nose data in many applications can be inadequate. Olive oil quality as one of the most important edible oils around the world has been considered by fusion of e-noses and tongues. In a study, an electronic nose and electronic tongue fusion system coupled with multivariate techniques have been successfully employed to recognize the geographical origin and the distinctiveness of specific extra virgin olive oils (Cosio et al., 2006). Experiments were performed with a commercial electronic nose that consisted of three parts: an automatic sampling apparatus, a detector unit including the array of sensors, and software (Senstool) for data recording and processing. The automatic sampling system provides a carousel of 12 sites for loading the samples and allows controlling the internal temperature. Twenty-two different sensors were included in the sensor array: 10 sensors were metal oxide semiconductor field effect transistors (MOSFET) and 12 were Taguchi type sensors (metal oxide semiconductors—MOS). Also, the electronic tongue system was developed based on the flow injection analysis (FIA) with two amperometric detectors. The detection units of the electronic tongue consisted of a reference (Ag/AgCl saturated) electrode, a working electrode (a dual and a single glassy carbon electrode, respectively), and a platinum counter electrode. In the flow system developed, a carrier solution is continuously pumped through the amperometric detectors and the olive oil samples are then injected into the flow stream. Amperometric detectors present in the system provide the oxidation of electroactive compounds at the working electrode, while a constant potential is applied. They concluded that the fusion system could show a reliable recognition device for the classification of the geographical origin of extra virgin olive oils from a restricted area. The classification model developed by the selected electronic nose sensors seems more reliable than the model developed with all the variables, both for cross-validation and test results. Based on their results, the classification model built with the electronic tongue sensors provides suitable results, but its prediction capabilities are worse than those of the electronic nose.

In another study on olive oil, Cosio et al. (2007) tried to find the feasibility of the classification of olive oil samples stored in different conditions and periods using the electronic nose and electronic tongue fusion system combined with chemometrics tools. Chemical parameters and electronic tongue data were not correlated in the linear discriminant analysis model. Indeed, it was revealed that classification accuracy is preserved by removing chemical analysis and electronic tongue sensors leading to more applicable model. According to the results reported, the electronic nose data was used to build the final classification model. The model could describe the olive oil storage conditions and show simple and fast classification tool. The authors found the fusion system to be promising in olive oil industry applications.

The first work on the fusion of three senses (taste, odor, and vision) to olive oil characterization was reported by Apetrei et al. (2010), who conducted a work to recognize the organoleptic characteristics of 25 extra virgin olive oils from different varieties and with different bitterness degree. For this purpose, an electronic panel comprising an electronic eye, an electronic nose, and an electronic tongue was developed. In the case of the electronic eye, the transmittance spectra were recorded using a series of light-emitting diodes (LEDs) covering the range from 780 to 380 nm and for the electronic tongue measurements from carbon paste electrodes modified with olive oils were fabricated, while 13 MOS-based sensors were selected and introduced in sensor chamber. As performed in experimental protocol, 2 g of the olive oil sample were placed in 10 mL vials. Then, the vials were thermostated at 40°C during 15 min in slow agitation, followed by an equilibrium stabilization step of 10 min. A representative sample of the headspace was collected using an automatic system and injected into the sensor chamber using a carrier gas flow (synthetic air at 100 mL/min). The sensor chamber was maintained at a constant temperature (50°C) and under a constant flow of synthetic air. The resistance changes were recorded using a data acquisition card. The data obtained from the electronic nose, the electronic tongue, and the electronic eye were then fused and analyzed. According to the report, individual data did not show good results, but after data fusion, satisfactory characterization was found among the VOO samples. Fig. 27.2 shows the responses of individual systems toward some VOO samples and some results of data analysis.

Fusion of the electronic nose, the electronic tongue, and the electronic eye was later performed to analyze other food products (Ouyang et al., 2014).

In another research effort, a low-cost electronic nose was fused with a simple electronic tongue system to recognize the five virgin olive oils from different geographical areas of Morocco (Haddi et al., 2013). The electrochemical experiments were accomplished in a usual electrochemical cell comprising a three-electrode system and an electronic nose including a 5 MOS sensor array was used. The signals and voltammograms were recorded as illustrated in Fig. 27.3.

The data of both electronic systems were extracted and then fused. A nonlinear chemometric technique called the support vector machine (SVM) was addressed for data analysis. As mentioned in their report because of the relatively small number of measurements, leave-one-out cross-validation approaches were implemented to estimate the true success rate that could be achieved with the SVMs. The results showed satisfactory distinct classification.

## 27.4 FUTURE TRENDS AND PERSPECTIVE

Electronic tongues and noses are generally comprised of a few to tens of sensors. Reducing the number of the sensors and relevant methods have been underscored earlier (Ciosek et al., 2004). By reduction of the irrespective variables, the sensor array can improve the discrimination capability among different groups of the samples. Quantitative arrangement of recognition capability of the array before and after reducing of number of the sensors is necessary to carry out further classification tasks. This approach should be addressed in future studies and the results could be used in any fusion of electronic noses and tongues to characterize the quality of olive oils with high success rates.

Application of electronic noses and tongues are very helpful in the olive oil industry. Nevertheless, the detection capability of the sensors primarily is influenced by adsorbability or catalysis of those sensitive materials to particular odors and ions. Although important achievements have been reported, this approach still has restrictions in sensitivity and specificity, in comparison with the biology binding of specific odorants and tastants to the olfactory and taste receptor cells (Pearce, 1997). Thus, the study of fusion of the olfactory and taste is still at an early stage. Only a few kinds of electronic nose and electronic tongue fusion systems are in commercial use. For that reason, the research on fusion of artificial olfactory and taste systems is still very important for the future development.

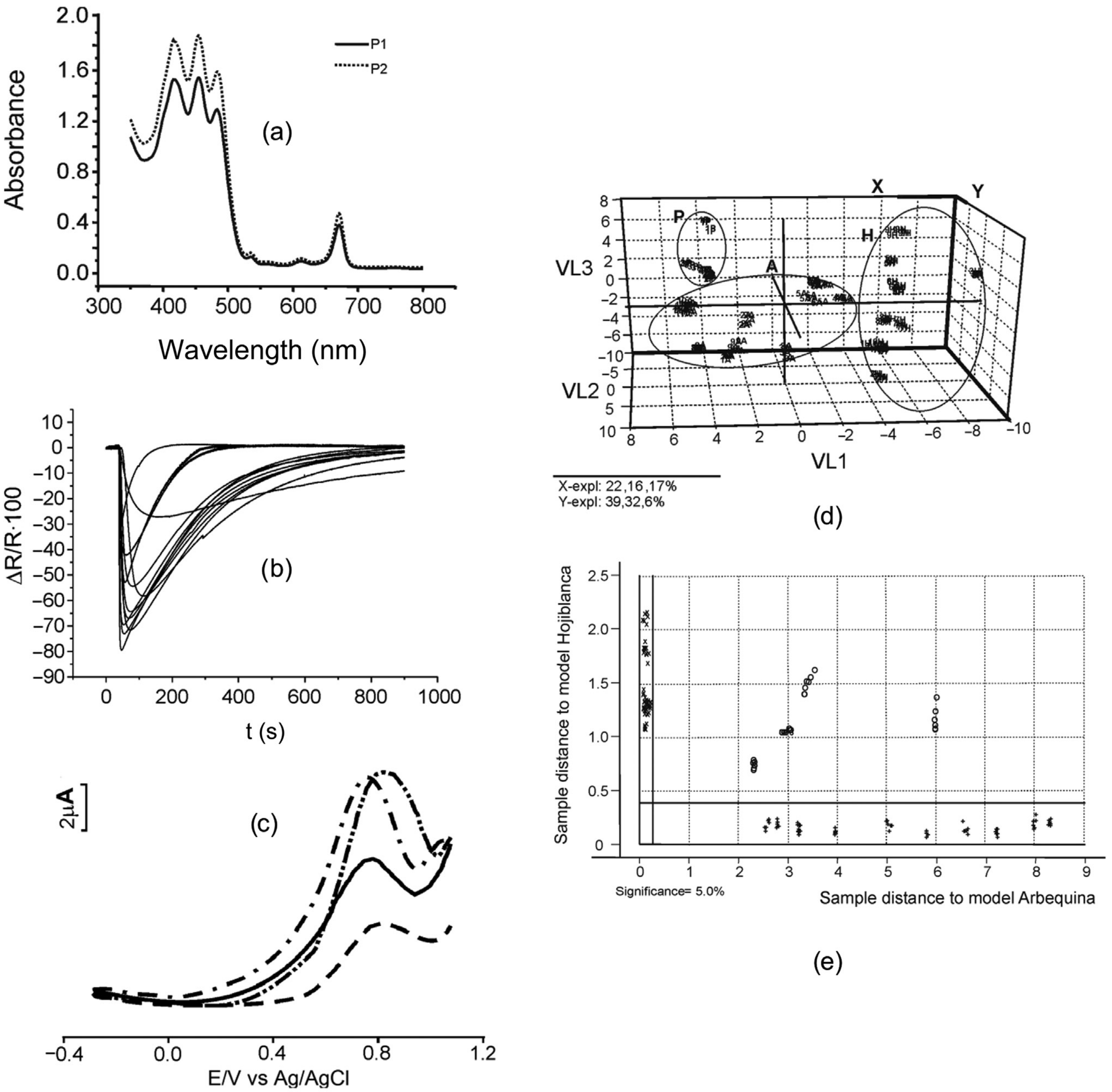


**FIGURE 27.2** (a) Electronic spectra of Picual samples P1 and P2; (b) Sensor array response in the presence of aromas of the sample A5; (c) Square-wave voltammetric curves (forward scan) of sensors modified with Picual samples P1 *(•–•–•)*, P2 *(- ••-•• -)*, P3 *(—)*, and P4 *(- - -)* immersed in aqueous solution 0.1 mol/L KCl; (d) PLS-DA scores plot of data fusion corresponding to the classification of oil according their olive variety; (e) Coomans plot from SIMCA classification. The classes modeled are oil belonging to Hojiblanca and Arbequina. The solid lines accounts for the 95% probability boundaries; "*o*" test samples, Picual; "+" model samples, Arbequina; "×" model samples, Hojiblanca.

Online fusion of electronic nose and electronic tongue can play a key role in the automation of olive oil quality evaluation and control. In the near future when the basic challenges of the fusion sensors have been solved, we will see more online electronic nose and electronic tongue fusion systems in the olive oil industry. For each application, however, technical problems have to be rectified for implementation of online fusion systems. Because, in some cases, electronic nose and electronic tongue fusion systems cannot get complete information on the olive oil quality indicators, a combination of different approaches may provide a robust method of olive authentication. In this regard, richer information is obtained to augment the capability of the system. Thus, the electronic nose and electronic tongue fusion system coupled with innovative instruments would probably provide an effective method to olive oil quality

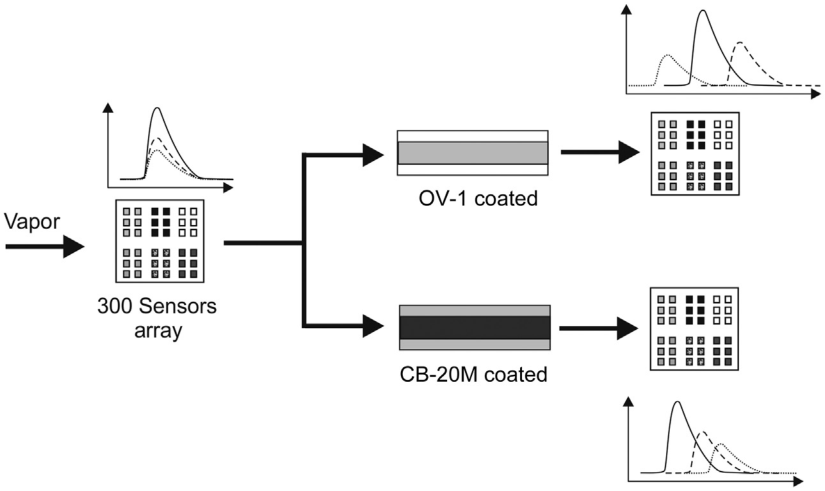


**FIGURE 27.3** **Dual-column concept with large sensor arrays OV-1, nonpolar stationary phase; CB-20M, Carbowax, a polar stationary phase.** *(With kind permission from Che Harun et al., 2009.)*

authentication (Ghasemi-Varnamkhasti et al., 2010). More recently, an innovative and emerging type of electronic nose and tongue, that is, a bioelectronic nose and a bioelectronic tongue, were introduced in which biosensors as sensing elements are employed (Ghasemi-Varnamkhasti et al., 2011).

A novel kind of an electronic nose called an electronic mucosa that tries to mimic nasal chromatograph effect with more useful information content in such a way that a higher level of recognition compared with the existing electronic nose systems could be developed. A typical electronic mucosa as an innovative instrument is shown in Fig. 27.4, in which three large arrays of sensors with a two retentive columns have been combined (Che Harun et al., 2009).

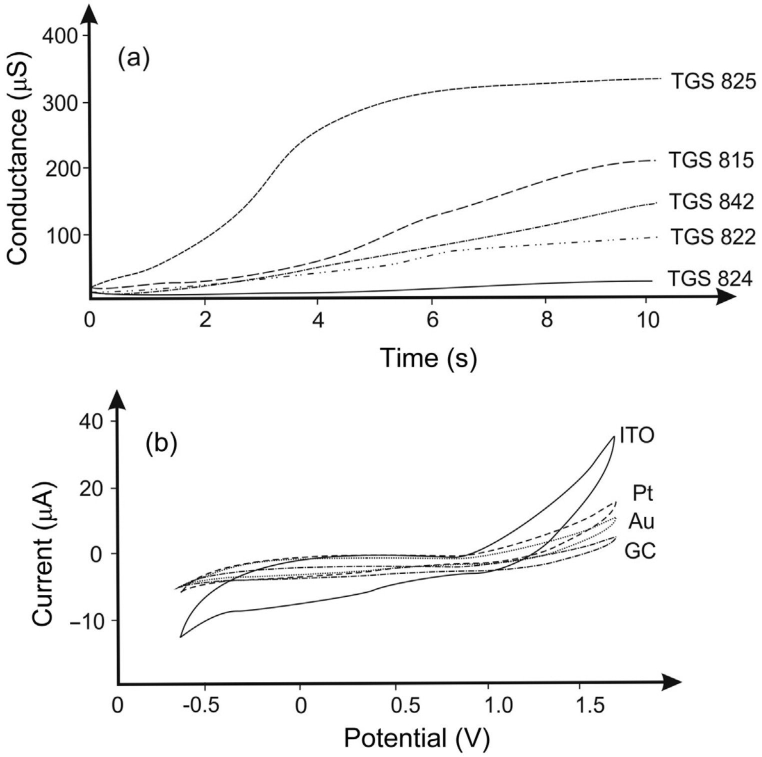


**FIGURE 27.4** (a) Time responses of the electronic nose used with an array of five TGS (815, 822, 824, 825, 842) sensors toward exposures to the VOO volatiles; (b) Cyclic voltammograms obtained by electronic tongue used with the four electrodes; ITO, indium tin oxide; Pt, platinum; Au, gold; GC, glassy carbon. *(With kind permission from Haddi et al., 2013.)*

In e-mucosa, all sensors give spatial information as done in a regular electronic nose, but with many sensors. The second and third arrays give other temporal profiles, which are different from each other and the first array (Ghasemi-Varnamkhasti and Aghbashlo, 2014). In electronic mucosa, when an odor signal passes through down a microchannel, an absorbent coating provides a delay akin to the work of the mucous layer in the nasal cavity. This absorbent material selectively does a delay in the odor signal, consequently, the generation of an odor/coating certain time delay. It is worth noting that the partitioning impact is to some extent such as a traditional gas. Until now, no application of electronic mucosa has been reported to olive oil analysis applications. This device can be addressed for olive oil quality assurance and control to provide more effective information on the oil quality indicators. Data fusion as an advanced approach can also be considered to get more complete and accurate information compared with individual sources of data (Aghbashlo et al., 2014). However, advanced chemometric tools should be included in the system to analyze the data gathered (Brereton, 2007; Otto, 2007). Such an idea might be practical in olive oil industries in the near future.

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## Chapter 28

# Alcoholic Fermentation Using Electronic Nose and Electronic Tongue

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## 28.1 INTRODUCTION

Fermentation is one of the oldest preservative technologies with the aim to ensure that food is maintained at an acceptable level of quality from the time of manufacture to the time of consumption. Fermentation, as a food preservation technique, can be traced back to thousands of years ago. It is thought that the art of cheese making was developed as far back as 8000 years ago between Tigris and the Euphrates rivers in Iraq (Fox, 1993). Alcoholic fermentation involved in winemaking and brewing developed during the period 2000–4000 BC by the Egyptians and Sumerians.

Food fermentation has four main purposes: the development of a diversity of flavors, aromas, and textures in food substrates; the preservation of food through the development of lactic acid, alcohol, and acetic acid; the biological enrichment of food with proteins, essential amino acids, and vitamins; and the elimination of antinutrients.

Alcoholic fermentation is a complex biochemical process involving interactions between yeasts, bacteria, and fungi. During the alcoholic fermentation yeasts utilize sugars (glucose and fructose) and other constituents as substrates for their growth, converting these to ethanol, carbon dioxide, and other metabolic byproducts that contribute to the chemical composition and sensory quality of the fermented foodstuffs (Graham and Gilliam, 1993). Alcoholic fermentation is the basis for the manufacturing of alcoholic beverages such as wine and beer.

Wine is a natural product resulting from a number of biochemical reactions, which begin during ripening of the grapes and continue during harvesting, alcoholic fermentation, clarification, and bottling. Many of these reactions are due to a large variety of molds, bacteria, and yeasts present on grapes' surface (Torija et al., 2001).

Apart from the principal wine yeast, *Saccharomyces cerevisiae*, spontaneous alcoholic fermentation is a complex process carried out by the sequential action of different yeast genera and species, found on the grapes, in the must, and in the wine (Heard and Fleet, 1988).

Beer is the most popular alcoholic beverage, and probably one of the oldest manufactured by humans. The brewing process is based on the fermentation of starch, commonly derived from cereal grain such as barley, wheat, maize, and rice, in part malted. Most beers are flavored with hop (*Humulus lupulus*), which adds bitterness and aroma also acting as a natural preservative (De Keukeleire et al., 1992; Tanimura and Mattes, 1993).

The byproducts of fermentation are aromas and flavors, whose evaluation is very important since they determine the quality of alcoholic beverages, positively or negatively affecting their sensorial properties. The flavor of alcoholic beverages is composed by a very large number of compounds; more than 1000 volatile compounds have been identified and most of them are produced by yeasts during fermentation (Nykänen, 1986).

Wine flavor includes compounds originating from the grapes (varietal flavor), compounds formed during the extraction and conditioning of must (prefermentative flavor), other compounds produced by yeasts and bacteria during alcoholic and malolactic fermentation (fermentative flavor), and compounds produced during the aging process (postfermentative flavor), as reviewed by Schreier (1979), Boulton et al. (1995), and Rapp (1998). Volatiles identified in wine are usually dominated by the main fermentation products: ethanol and carbon dioxide, which contribute little to wine flavor, conversely organic acids, higher alcohols, esters, and to a lesser extent acetaldehyde constitute the main group of compounds that form the "fermentation bouquet" (Rapp and Versini, 1991).

In a highly competitive market, wineries and brewers need to invest more in technology to increase productivity and to optimize product quality. Control of fermentation is generally considered as a prerequisite to determine the quality of alcoholic beverages; in this context, fermentation

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monitoring is a growing need, which calls for fast methods providing real-time information in order to assure an effective control at all stages of the process.

Online fermentation monitoring is a promising method for improving fermentation control because it is much more accurate than manual measurements and it makes possible new control strategies in which the operative conditions are adapted to the fermentation proceeding. Several methods for online fermentation monitoring have been proposed: density measurement (El-Haloui et al., 1988), ethanol concentration (Warriner et al., 2002), and $CO_2$ produced (Corrieu et al., 1997). Some authors have tried to develop methods for measuring specific fermentation products using biosensors (Mello and Kubota, 2002; Mao et al., 2008). Others have investigated the feasibility of simultaneous monitoring of several products by Fourier transform–infrared spectroscopy (FT–IR) (Zeaiter et al., 2006).

One of the most promising directions for the development of rapid, low-cost, and nondestructive methods is the application of sensor systems, whose speed and online capabilities meet the demand of automation and continuous process control. Electronic nose (e-nose) and electronic tongue (e-tongue) are technological attempts to mimic the function of human senses. Both devices consist of a chemical sensor array coupled with an appropriate pattern recognition system able to interpret complex signals from sensors and produce a fingerprint of the product. The strength of both devices include their high sensitivity and that they are easy to use. Many applications of e-nose and e-tongue to monitor the fermentation processes have been published; some of them are interesting reviews (Escuder-Gilabert and Peris, 2010; Peris and Escuder-Gilabert, 2013; Rudnitskaya and Legin, 2008). One in particular describes the application of an e-nose in brewery for beer quality assessment (Ghasemi-Varnamkhasti et al., 2011). Some works reported in literature concern the application of the e-nose and the e-tongue to alcoholic fermentation monitoring. The main features of these applications are reported in Table 28.1.

**TABLE 28.1** Main Application of E-Nose and E-Tongue in Alcoholic Fermentation Monitoring

| | Samples | Aim | Sampling | Sensors | Data Analysis | References |
|---|---|---|---|---|---|---|
| E-nose | Wine-must | Monitoring of aroma during fermentation | Online–Offline | 32 CPs (A32S AromaScan) | PCA | Pinheiro et al. (2002) |
| | | Process control in wine fermentation | Offline | 6 Taguchi sensors by Figaro | PCA | Maciejewska et al. (2006) |
| | | Discrimination of partially fermented wines | Offline | 8 QMB | PCA | Garcia-Martinez et al. (2011) |
| | | Quantification of alcohol during red wine fermentation | Offline | 10 MOS | PCR and PLSR | Zhang et al. (2012) |
| | | E-nose development for fermentation monitoring | Offline | 2 MOS—1 electrolyte sensor (Wi-Nose) | ANN | Linehan et al. (2010) |
| | | Wine spoilage caused by *Brettanomyces* | Offline | MS (Hewlett Packard) | PCA, PLS, and SLDA | Cynkar et al. (2007) |
| | | Comparison of e-nose and MS e-nose for *Brettanomyces* spoilage | Offline | MS (Hewlett Packard) and 12 MOS (Fox 3000) | PLS | Berna et al. (2008) |
| | Synthetic media containing *S. cerevisiae* | Monitoring of physiological changes in fermentation processes | Online | 4 MOS and 10 MOSFET | ANN and PLS | Bachinger and Mandenius (2001) |
| | | Identification of two strains along alcoholic fermentation | Online | 18 MOS (Fox 4000) | PCA and DFA | Calderon-Santoyo et al. (2010) |
| | Beer | Monitoring of draft beer fermentation process | Offline | 7 MOS | PCA | Phechakul and Sutthinet (2014) |
| E-tongue | Beer | Monitoring of beer fermentation | Offline | 10 Potentiometric and 3 voltammetric sensors | PLS–DA and MLR | Kutyla-Olesiuk et al. (2012) |
| E-nose and E-tongue | Wine-must | Monitoring of wine-must fermentation | Offline | EDU/10 MOS (PEN2), lipidic sensor (SA 402B) | PCA | Buratti et al. (2011) |

## 28.2 E-NOSE APPLICATIONS

Several works reported in Table 28.1 concern the application of the e-nose to the monitoring of flavor and aroma compounds produced during the alcoholic fermentation process; as can be observed, electrochemical sensors, mainly metal oxide semiconductor (MOS), metal oxide semiconductor field-effect transistor (MOSFET), and conducting polymers (CPs), are the most used. Additionally, the mass spectrometry (MS) based e-nose has been used in this research area. Regarding the data processing, classical statistical methods such as principal component analysis (PCA), artificial neural network (ANN), and partial least square (PLS) regression are widely used.

Pinheiro et al. (2002) studied the aroma production during wine-must fermentation by means of a commercially available e-nose with an array of 32 organic CPs sensors. This work discussed the e-nose feasibility for the online and real-time monitoring of the muscatel aroma evolution during fermentation. The authors demonstrated that without sample pretreatment the e-nose could only perceive the ethanol production. By a selective enrichment step using organophilic pervaporation, the e-nose was able to detect the aroma compounds even in presence of ethanol, making it possible to discriminate between samples according to their aromatic fingerprint.

A similar work was performed on Tokaj wine fermentation monitored with gas sensor measurements, traditional instrumental methods, and human sensory evaluation (Maciejewska et al., 2006). Gas sensor measurements were performed with a homemade device consisting of 6 Taguchi gas sensors. The collected data were processed with PCA and the first principal component (PC1) scores were found highly correlated with alcohol content, volatile acidity, and to a less extent with ethylacetate concentration in fermented medium. Additionally, the PC1 scores strongly correlated with human sensory assessment of odor and flavor produced during wine fermentation. Therefore, the potential of gas sensor array for providing useful information for wine fermentation control was pointed out.

An interesting contribution (Garcia-Martinez et al., 2011) deals with the discrimination of partially fermented sweet wines by means of gas chromatographic (GC) and e-nose analyses. The e-nose was developed and assembled at the University of Tor Vergata (Rome) and the array was composed of 8 quartz microbalances (QMBs) sensors. Fermentation tests were conducted with two *S. cerevisiae* strains selected for their tolerance to high osmotic pressures and ethanol concentration. PCA applied to e-nose data allowed the discrimination between fermented and unfermented musts, but the discrimination between wines obtained by the two selected *S. cerevisiae* strains was not possible. PCA applied to GC data showed a clear discrimination between wines produced by the two yeast strains when 2,3-butanediol and glycerol were removed from the PCA elaboration.

Near infrared (NIR) spectroscopy and e-nose were used to predict the alcohol production during red wine fermentation (Zhang et al., 2012). Calibration models were developed between instrumental data and chemical analyses using the principal component regression (PCR) and the partial least squares regression (PLSR). Good correlations ($r > 0.99$) were obtained for both the models developed on NIR and e-nose data. Combining NIR and e-nose, the model can be optimized and the prediction accuracy improved. Although the measurements were carried out in offline mode, this study demonstrated that NIR spectroscopy and e-nose can be used as online, fast, and nondestructive techniques to provide in-time information about the fermentation process and to assure the quality of final products.

Linehan et al. (2010) developed an e-nose, called Wi-Nose, to provide the wine industry with a lightweight, compact, and accurate sensing device for wine fermentation monitoring. The sensor array was composed of 3 sensors: 2 MOS sensors for the detection of ethanol and 1 electrolyte sensor for the detection of carbon dioxide. Wi-Nose was successfully designed to examine the fermentation with the help of ANN. The goal was to train the neural network to classify the fermentation in three stages: the first stage or aerobic fermentation, the second stage or anaerobic fermentation, and the third stage or malolactic fermentation. The best training resulted in an accuracy of 100% for all stages.

During the fermentation process, unpleasant taints are produced by *Brettanomyces* yeasts spoilage. The two components of the taint are 4-ethylphenol (4EP) and 4-ethylguaiacol (4EG). Typically, the taints are described as "barnyard," "sweaty saddle," and "Band-aid," when present in the red wine at concentration of several hundred micrograms per liter. The existing procedure to evaluate the spoilage due to *Brettonomyces* are time consuming and expensive, so there is a need for a rapid and low-cost screening method to monitor this type of spoilage. In this way Cynkar et al. (2007) used a MS-based e-nose to monitor the *Brettanomyces* spoilage in 213 Australian red wines. The MS e-nose data elaborated by chemometric methods, such as PCA, PLS, and stepwise linear discriminant analysis (SLDA) resulted in an acceptable discrimination between spoiled and unspoiled wines. Furthermore, Berna et al. (2008) compared the performance of two techniques, the MOS sensors based e-nose and the MS e-nose, for the prediction of *Brettanomyces* spoilage on 46 Australian red wines purchased from retail outlets. GC–MS was used for quantification and prediction purposes. Following ethanol removal and solid-phase microextration (SPME) sample handling, the detection limits of MOS e-nose were 44 mg/L for 4EP and 98 mg/L for 4EG; these values were significantly lower than the human sensory thresholds. PLS regression of e-nose data against known levels of 4EP and 4EG showed that MOS e-nose was unable to identify

*Brettanomyces* spoilage reliably because of the response of MOS sensors to inter-sample variation in VOCs other than 4EP and 4EG. The MS e-nose performed significantly better than the MOS e-nose in quantifying ethylphenols, and its performance is acceptable for concentrations of 4EP higher than 20 mg/L. Correlation coefficients ($r$) of 0.97 and 0.98 were obtained between concentrations of 4EP and 4EG estimated by MS e-nose and by conventional GC–MS.

Bachinger and Mandenius (2001) presented an online approach for noninvasive monitoring of physiological changes in fermentation processes using an e-nose equipped with 10 MOSFET sensors, 4 MOS sensors, and 1 infrared (IR) sensor measuring $CO_2$ concentration. The responses of gas sensors in monitoring the composition of the bioreactor headspace produced by two different cultures (*S. cerevisiae* and *Escherichia coli*) are used to obtain a semiquantitative representation of the physiological state of the cultures. E-nose data showed that physiological variables such as growth rate, substrate uptake, and product formation can be monitored. Because of the semiquantitative nature of the approach, the method is not suited for process development purposes. However, the possibilities of an online and nondestructive measurement procedure make it a simple and fast method for the monitoring of industrial bioprocesses.

A commercially available e-nose composed with 8 MOS sensors was tested to monitor alcoholic fermentation of two *S. cerevisiae* strains (ICV-K1 and T306), well known for their use in enology (Calderon-Santoyo et al., 2010). Samples were dealcoholized by a patented back-flush technique (Ragazzo-Sanchez et al., 2004), and the e-nose was applied to investigate online alcoholic fermentation and to discriminate the two different yeasts. The two strains were characterized by a very similar tendency in biomass or ethanol production during fermentation. The e-nose was able to establish a kinetic of the aroma production that can be associated with the fermentation phases. The PCA of e-nose responses appeared to be mainly influenced by fermentation phases. In particular, the PCA score plot showed three different clusters: the first one is composed by the measurements carried out during the lag and fast growth phases, the second was attributed to the main aroma compounds release stage, and the third one was related to the progressive deceleration phase. After a specific data treatment limiting the influence of time, discriminant factorial analysis (DFA) was carried out on normalized data and was able to clearly identify differences between the two yeast strains and the overall performance achieved was 83.5%.

Finally, Phechakul and Sutthinet (2014) monitored beer fermentation by an e-nose composed of 7 MOS sensors. The fermentation process of Thailand's commercial draft beer was studied in this work. Measurements were performed every hour for 10 days and the PCA technique was used for data processing in order to evaluate the aroma evolution during the fermentation period. The proposed system would be useful to ensure a quality standard of the production process.

## 28.3 E-TONGUE APPLICATIONS

E-tongue has been successfully applied to the monitoring of several components produced during the fermentation processes. Regarding sensors, the most used are potentiometric (especially ion-selective electrode based on PVC membranes, chalcogenide glass, and artificial lipid membranes) and voltammetric (Peris and Escuder-Gilabert, 2013; Rudnitskaya and Legin, 2008). Only a few works regarding the alcoholic fermentation monitoring can be found in literature (Table 28.1). Therefore, it could be useful to review e-tongue applications on other types of fermentation processes since such applications are more numerous, the analytical approach is similar, and the produced compounds are often the same.

An e-tongue comprising 21 potentiometric sensors with both chalcogenide glass and PVC plasticized membranes was used for offline measurements of batch *E. coli* fermentations (Turner et al., 2003). The e-tongue was able to monitor the changes in media composition as fermentation proceeded; in particular, the increase in organic acids, especially acetic acid, was detected and the biomass dry weight was predicted with good accuracy.

A potentiometric e-tongue consisting of 8 chemical sensors based on PVC plasticized membranes with enhanced cross-selectivity to inorganic cations and organic acid anions, and a standard pH electrode was applied to analyze a simulated media for *Aspergillus niger* fermentation (Legin et al., 2004). The e-tongue was able to evaluate ammonium, citrate, and oxalate content in simulated media similar to real samples involving *A. niger* fermentation. Data processing using ANN provided average prediction errors lower than 8%. ANN produced better results than PLS in the data fitting for ammonium and citrate concentration, probably due to the nonlinear dependence between sensor potentials and concentration.

Imamura et al. (1996) used a multichannel taste sensor system with 8 different lipidic membranes for monitoring the changes in the taste of miso (Japanese soybean paste) during the fermentation process. It was found that the response of two sensors increased linearly with the number of days of miso fermentation. Moreover, satisfactory correlation coefficients ($r > 0.87$–$0.88$) between the sensor output and two chemical parameters (amino acid contents and titrable acidity) were obtained.

In the work by Kim et al. (2005), a homemade multichannel taste sensor comprising of 8 potentiometric chemical sensors with PVC plasticized membranes was applied to the monitoring of Kimchi fermentation (a Korean traditional pickle fermented with lactic acid bacteria). Samples were matured for 10 days at three storage temperatures

(4, 10, and 25°C) and during fermentation titrable acidity, which is a maturation index of various pickles, was determined. It was found that the sensor responses increased during the fermentation period at the three storage temperatures. When the sensor data were elaborated by PCA, the PC1 scores were mostly correlated with the values of titrable acidity.

An e-tongue based on 30 potentiometric chemical sensors with chalcogenide glass and solvent polymeric membranes was applied to the monitoring of a batch fermentation process of a starting culture for light cheese production (Esbensen et al., 2004). Process control charts were built on the e-tongue responses by using PLS regression. The control charts allowed the detection of fermentations running under "normal" and "abnormal" operation conditions. Moreover the e-tongue capability to quantify organic acids (citric, lactic, and orotic) in the fermentation media was demonstrated with average prediction error in the range of 5–13%. The correlation between peptide profile determined by HPLC and e-tongue data was also established.

Regarding the alcoholic fermentation, Kutyla-Olesiuk et al. (2012) monitored beer fermentation by using a hybrid e-tongue combining both potentiometric and voltammetric sensors. The applied sensor array consisted of 10 miniaturized ion-selective sensors and silicon-based 3-electrode transducers. The analysis was performed on homemade beer during two stages of production: fermentation and beer maturation. The obtained results were processed by PLS and PLS–discriminant analysis (PLS–DA). For potentiometric data, voltammetric data, and combined potentiometric and voltammetric data, comparison of the classification ability was conducted. The obtained results demonstrated that the developed hybrid e-tongue (combination of potentiometric and voltammetric sensors) provided lower classification error of samples according to their fermentation and maturation time.

## 28.4 E-NOSE AND E-TONGUE APPLICATIONS

The monitoring of time-related changes occurring during alcoholic fermentation was investigated by Buratti et al. (2011) and Buratti and Giovanelli (2011). In these works infrared spectroscopy, in both near and mid regions (FT-NIR and MIR spectroscopy), e-nose and e-tongue were applied and combined with multivariate statistical methods to monitor red wine fermentation in order to classify samples on the basis of their fermentation stages and to predict chemical parameters.

Eight microfermentation trials were carried out at controlled temperature using active dry yeast inoculum (*S. cerevisiae*) and were conducted in Valtellina region (Northern Italy) during the 2008 and 2009 vintage, on Nebbiolo grapes ecotype Chiavennasca. For each trial, samplings were performed at subsequent times during fermentation. In order to follow the evolution of chemical parameters during must-wine fermentation, glucose, fructose, ethanol, and glycerol were evaluated by HPLC. The spectroscopic techniques were used to investigate molecular changes, while e-nose and e-tongue evaluated the aroma and taste profile during alcoholic fermentation.

E-nose measurements were performed with a commercial device, operating with an array of sensors, combined with the enrichment and desorption unit (EDU), a microprocessor-controlled device capable of automatically trapping and thermally desorbing the sample headspaces and able to remove the major volatile compounds not important for odor (ie, $CO_2$ and ethanol). The sensor array was composed of 10 metal oxide semiconductor (MOS) type chemical sensors: W1C (aromatic), W5S (broadrange), W3C (aromatic), W6S (hydrogen), W5C (aromatic-aliphatics), W1S (broad-methane), W1W (sulfur-organic), W2S (broad-alcohol), W2W (sulfur-chlorinate), and W3S (methane-aliphatics).

E-tongue analysis was performed with a commercial taste-sensing system. The detecting part consisted of working sensors whose surface is combined with artificial lipid membranes having different response properties to chemical substances based on their taste.

During microfermentation trials, sugar consumption and ethanol and glycerol production were modeled by the Gompertz equation (Zwietering et al., 1991) in order to follow the kinetics of fermentation parameters.

PCA was applied to spectral, e-nose and e-tongue data, as an exploratory tool, to uncover modifications during fermentation. Linear and quadratic discriminant analysis (LDA and QDA) were applied in order to obtain classification models. Genetic algorithms (GA) were used to select the subset of spectral ranges, e-nose and e-tongue variables that maximize the predictive power of the classification models. When dealing with NIR and MIR spectra, discriminant analysis was calculated on PCA scores.

PCA results on NIR and MIR spectral data showed a satisfactory distribution of the samples according to the fermentation time for each microfermentation trial. The intensity loadings put in evidence that the main wavenumbers responsible of the sample separation were associated with the combination band of C–H (4454–4250 $cm^{-1}$) related to carbohydrates in the near region, and with the C–O and C–C bonds (1087–1045 $cm^{-1}$) of ethanol and carbohydrates in the medium region.

The evolution of the taste and aroma profile during fermentation was evaluated by e-tongue and e-nose. Fig. 28.1 shows the PCA score plot (a) and the loading plot (b) of the data collected by e-tongue on a microfermentation trial.

Considering the score plot (Fig. 28.1a), the taste evolution is evident along the PC1, must-wine samples are distributed from left to right according to the fermentation time. By the loading plot (Fig. 28.1b), it is evident that at

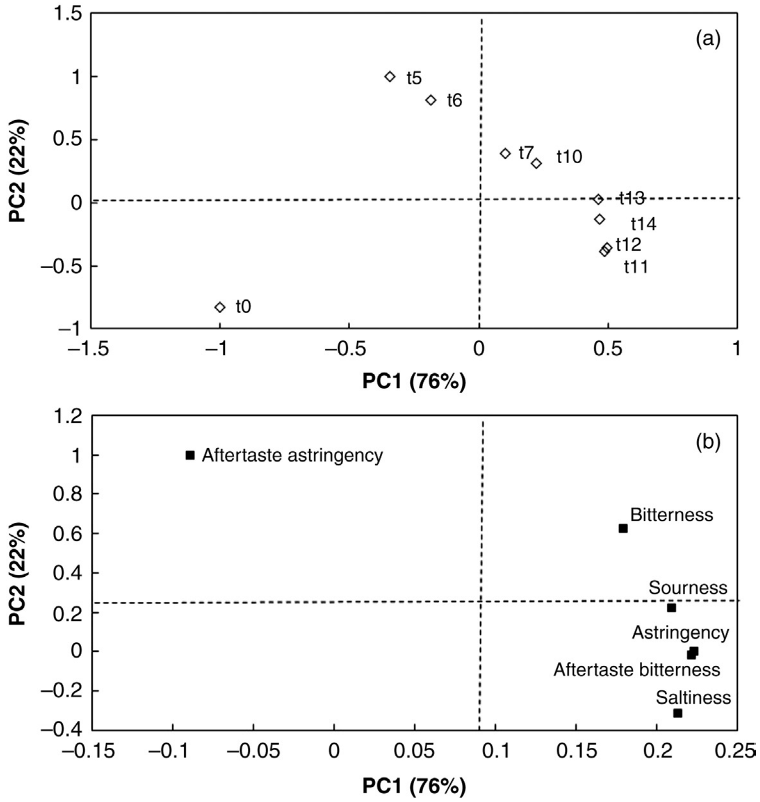

**FIGURE 28.1 PCA on e-tongue data.** (a) Score plot and (b) loading plot of a microfermentation trial in the plane defined by the first two principal components.

the beginning of process (t0) sample was characterized by a poor taste; during fermentation (t5–t6) the taste evolves and samples were discriminated by the aftertaste astringency; at the end of fermentation (t10–t14), wine samples were perceived as more bitter and astringent and were also characterized by saltiness and sourness. The taste of red wines depends on the phenolic compounds contained in the grape skin and seeds. During alcoholic fermentation, anthocyanins, flavan-3-ols, and tannins are transferred to wine by diffusion and the extraction rate depends on their molecular size, relative solubility, and location in the berry. From the literature it is known that the increase of bitterness and astringency perceived throughout must-wine fermentation is mainly due to the diffusion of these compounds (Workman, 2001; Workman and Weyer, 2008). Furthermore, as evidenced by the e-tongue, the taste of wine is affected by nonphenolic components such as ethanol, glycerol, salts, and acids, which are formed or extracted during fermentation (Sims and Bates, 1994; Workman and Weyer, 2008).

Fig. 28.2 displays the PCA score plot (a) and loading plot (b) of e-nose data collected on a microfermentation trial.

The score plot (Fig. 28.2a) shows the distribution of must-wine samples along PC1 and PC2 according to fermentation time and the main fermentation stages. From the loading plot (Fig. 28.2b), it can be noticed that at the beginning of fermentation (t0) must volatiles were perceived by WC sensors specific in particular for the aromatic compounds. During the high fermentation rate (t5–t10), the aroma evolves rapidly and the evolution was perceived by WS sensors (W2S, W3S, W5S, W6S) characterized by a broad range sensitivity. Finally, at the end of fermentation (t11–t14), wine was discriminated by W1S and W1W sensors, which are sensitive to many terpenes and sulfur organic compounds. From the literature, it is known that the alcoholic fermentation generates the majority of volatile compounds present in wine: acids, alcohols, and esters (Gonzalez-Marzano et al., 2004).

For the classification analysis, all samples were divided into three stages according to the evolution of chemical parameters: beginning of fermentation, class 1; high fermentation rate, class 2; and end of fermentation, class 3.

Table 28.2 reported the characteristics of the classification models selected as the best (highest values of correctly classified samples in cross-validation) and the variables involved in each model.

For NIR and MIR data, QDA models were characterized by a high percentage of correct classification in validation

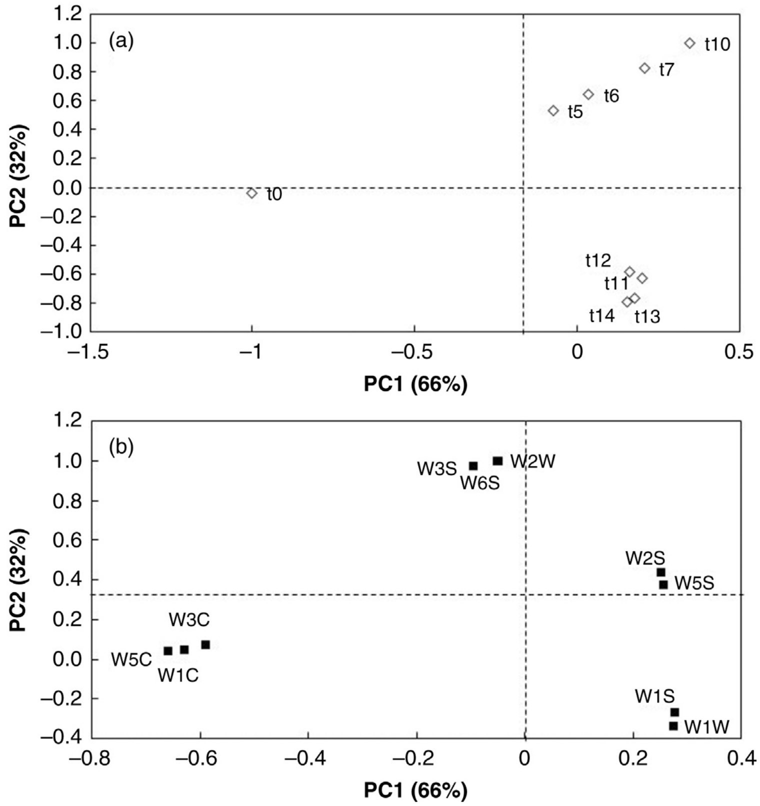

**FIGURE 28.2 PCA on e-nose data.** (a) Score plot and (b) and loading plot of a microfermentation trial in the plane defined by the first two principal components.

showing that the spectroscopic techniques are useful to predict the fermentation stages in agreement with the evolution of chemical parameters. The LDA model selected for e-tongue had an acceptable accuracy (84% of correct classification in validation), showing that the taste is an important parameter to define the quality of the product. Furthermore, the model is only composed by two variables, astringency and bitterness, useful to predict in a rapid and simple way the fermentation stage of must-wine samples and to give important information about the taste evolution. Even if the QDA model selected for e-nose had the lowest percentage of correct classification (76%), the e-nose device is useful to follow the evolution of the aroma profile during fermentation and to define the quality of wine.

In conclusion, the applicability of e-nose and e-tongue to monitor the alcoholic fermentation has been reviewed and discussed. The presented works demonstrated that these devices perform qualitative and quantitative determination

**TABLE 28.2** Characteristics of the Classification Models

| Applied Method | Model | NER[a] Cross-Validation (%) | Model Variables |
|---|---|---|---|
| NIR | GA-PCA-QDA | 93 | 5939.9–5905.2; 4485.8–4451.1; 4408.7–4373.9; 4370.1–4335.4; 4331.5–4296.8; 4254.4–4219.7[b] |
| MIR | GA-PCA-QDA | 91 | 2989.6–2970.3; 1396.4–1377.1; 1257.5–1238.3; 1188.1–1168.8[b] |
| E-nose | GA-QDA | 76 | W5C, W1W, W1S, W3S, W1C |
| E-tongue | GA-LDA | 84 | Astringency, Bitterness |

[a] *Non error rate.*
[b] *Spectral range (cm$^{-1}$).*

of compounds produced during fermentation and are suitable to follow the fermentation giving information about the quality of the final products. Moreover, these nondestructive techniques are of particular interest due to some important advantages respect to the classical analytical methods such as rapidity, simplicity, possibility of easy automation, and applicability for routine analysis also in online mode.

Although only a few works on the application of e-tongue and e-nose to the alcoholic fermentation monitoring can be found in literature, the presented results seem to be very promising to allow the continuous control of the fermentation process.

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Chapter 29

# Wine and Combined Electronic Nose and Tongue

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## 29.1 INTRODUCTION

Wine is a beverage obtained by fermentation of grapes and it represents one of the oldest alimentary products known by humanity for more than 8000 years. The first evidences of wine production have been found in Georgia and Iran, in the 6000–5000 BC historical period, and later the grape wine and winemaking culture spread from Egypt to China (McGovern, 2007). In some regions, the winemaking process has been applied to fruits other than grapes and in this case the drink obtained is called fruit wine, indicating what has been used for the production (eg, rice wine, widely distributed in the Asian region, apple wine, pomegranate wine, etc.).

It is interesting to note that wine is arrived nowadays almost without changes: while many foodstuffs have undergone variations in their ingredients and/or preparation procedures and some others have been completely left behind, with plenty of new formulations and food ingredients in use at present, the modern grape wine remains very similar to the one our ancesters knew. In fact, modern viticulture and oenology still refers to the ancient Greek practices, preserved and later evolved during the Roman empire.

The organoleptic properties of wine, such as the taste and flavor, are determined by a great number of wine components arising from grape and/or formed during the fermentation process. More than 800 compounds have been characterized in wine and their abundance of compounds depends on the grape variety and origin, on the viticultural practices, such as blending, specific aging, and, in particular, on the nature of yeast used in fermentation, as well as on the postproduction treatment and storage (Jackson, 2008). The standard analytical methods of wine analysis are based on wet chemistry procedures (Amerine and Ough, 1974; Ough and Amerine, 1988) or instrumental methods, involving separation techniques, such as high-performance liquid chromatography (HPLC) or gas chromatography (GC) combined with mass spectrometry (MS) (Flamini and Traldi, 2009), and optical techniques, such as Fourier transform infrared (FTIR) spectroscopy (Sun, 2009). For example, the WineScan™ FOSS analyser permits a rapid and accurate detection of a tenth of wine compounds optically active in the mid-infrared spectral region (www.foss.it). These techniques provide information on individual wine components, but they cannot give a global assessment, such as, the wine's flavor or quality, due to the synergistic interaction of several chemical compounds present in the complex chemical matrix represented by wine; moreover, the equipment applied is quite costly and requires calibration procedures, involving the employment of skilled personnel.

For this reason the involvement of a trained expert human panel remains indispensable in the wine analysis. However, the human perception of wine organoleptic characteristics is very subjective, determined not only by skills, but also by the mood and the physical state of the panelist. The number of tests that the panel may perform is limited to a few assessments per day, due to the saturation of tongue receptors. Moreover, the training of panelists is a long-lasting and expensive step.

The wine industry, however, requires to monitor time-related changes occurring during wine fermentation and aging to ensure the uniformity within a brand and to avoid falsifications. All these requirements can be fulfilled by the application of chemical sensor arrays mimicking the human senses, such as the electronic nose (e-nose), the electronic tongue (e-tongue), and recently the electronic eye (e-eye), which have demonstrated their utility for rapid and inexpensive assessment of different chemical matrices, and, among them, wine in particular (Lvova et al., 2013).

These devices complement the information obtained from the instrumental analytical methods (performed, for

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example, by LC–MS or GC–MS methods), since they cannot provide information about the exact composition of the analyte, but they can give a global evaluation of the analyzed matrix. Further advantages lie in a cheap and easy-to-operate analysis, often requiring little or no sample preparation, and they can be used in-line to obtain quick results, so allowing remediation procedures. With these characteristics, they can provide a rapid classification of samples of different quality or brand, and also the quantitative determination of several parameters.

Plenty of researches devoted to the application of these systems for wine analysis have been previously reported and several comprehensive reviews have been published in the past (Röck et al., 2008; Tahara and Toko, 2013; Bratov et al., 2010; del Valle, 2010). A comprehensive review on microtechnology-based hyphenated higher-order devices, employing sensors with different transduction principles integrated in the same sensing platform (but not fused for the same sensing layer), has also been reported (Hierlemann and Gutiérrez-Osuna, 2008), while a dual-mode sensing platform, where the same sensing material is exploited in the contemporaneous optical-potentiometric transduction, has been recently reported (Lvova et al., 2015).

More recently, the biological inspiration has created an approach that could boost the performances of these arrays in food analysis: in the flavor assessment humans apply as much as possible the combination of our senses to recognize the object of interest. In the same way, for chemical sensors the combination of several sensing techniques may significantly improve the resolving power of the resulting combined device, Fig. 29.1.

Moreover, the performance of the resulting analytical system may benefit of the employment of different transduction principles in the same device. In this way, the hybrid systems based on the simultaneous application of e-tongue, e-nose, and e-eye could provide sensitive and specific capabilities for a wide range of target analytes and unknown samples, which are currently difficult to detect with the existing technologies.

The correct exploitation of these combined systems requires an appropriate data analysis. Standard chemometric approaches, typical for multicomponent data treatment, are based on uniform and balanced data sets to guarantee the satisfactory treatment results. This aspect becomes particularly important in the case of combined sensor arrays, where the signals of the various sensors differ in terms of magnitude and unit of measurement and then they cannot be immediately compared. A method for the standardization of data is then necessary to ensure an equal treatment of the different sensor signals. A simple classical method is the normalization of the signals in order to reduce them to zero-mean and unitary variance.

The fusion of signals from different sensors can be approached from a different point of view. In particular, different levels of data fusion can be applied, these methods can be classified as a low level of abstraction, mid level of abstraction, and a high level of abstraction. Most often a low-level approach is used. This approach consists in the simple collection of data obtained by different sensors in order to form a single data matrix, where the number of rows is equal to the number of analyzed samples and the number of columns is equal to the total number of sensors signals (eg, potentials or currents and optical densities measured for every sensing layer) (Ruhm, 2007; Boilot et al., 2003).

In the mid-level fusion approach, the individual sensors are grouped in arrays and the signals are preprocessed in order to take advantage of the array properties. This method is naturally applied when sensor arrays, such as different electronic noses or electronic tongues, are used together in the same application. The most popular feature of the extraction method is the principal component analysis (PCA). In this case, the signals of the individual sensors of each array are replaced by the scores of the PCA.

The previous approach is further developed in the high level of abstraction where the data of each array are individually analyzed and then the results obtained by each model are combined together to provide a thorough

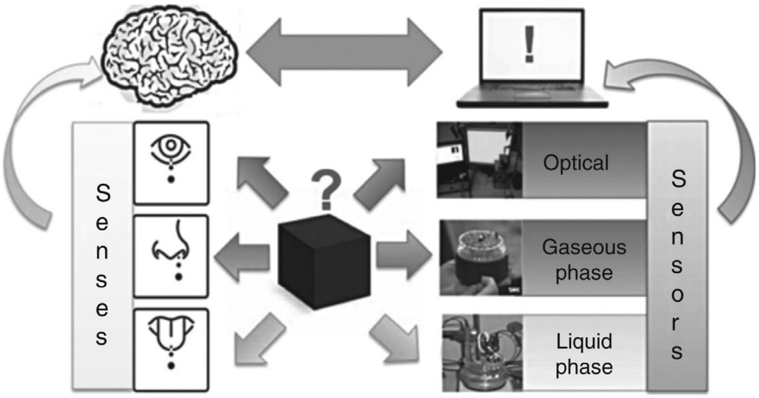

**FIGURE 29.1 The schematic presentation of human senses functioning versus chemical sensors approach.** *(Reprinted with permission from Lvova et al., 2015.)*

description of the measured sample. This approach seems to better mimic the working mechanism of natural senses that cooperate together to form a global perception of the experienced reality.

The exploitation of these combined systems in wine analyses is a field still not completely developed, although it is becoming more popular in the last few years. In Table 29.1, the different examples reported in the literature have been summarized and these studies are reviewed in this chapter, according to the different devices combined.

## 29.2 E-NOSE AND E-TONGUE

The seminal idea of combining e-nose and e-tongue devices for the analysis of complex chemical matrices started to be explored at the end of the 1990s (Toko et al., 1998; Wide et al., 1998; Winquist et al., 1999). Although one of the first works on combined e-nose and e-tongue was reported to be related on wine analysis (Rong et al., 2000), but it also analyzed other alcoholic beverages, the first application came from the collaboration of the Tor Vergata Sensor Group and chemical sensors laboratory of St. Petersburg State University (Di Natale et al., 2000). In this work, metalloporphyrin-based liquid and gas sensor arrays were exploited for both headspace and liquid phase analysis of red wine samples having the same denomination, Gutturnio, from a north Italy region.

The e-nose was constituted by an array of quartz crystal microbalances, coated by thin films of different metalloporphyrins deposited by the spray casting technique. The potentiometric e-tongue was formed by six polymeric membrane electrodes, using metalloporphyrins as ionophore, and a conventional glass pH electrode. The exploitation of the same sensing material gave the possibility to control the sensor characteristics for both phases. No sample treatment was necessary and the data obtained from the combined array were compared with those of the chemical analysis, with the aim to explore if some compound particularly influenced the sensor array responses. The data obtained clearly showed that the e-nose and e-tongue gave complementary information on the samples and that a satisfying estimation of several wine parameters could be obtained using the artificial sensor system.

In a subsequent work (Di Natale et al., 2004), a similar combined e-nose and e-tongue system was used to analyze 36 different wines produced in the 2001 vintage

**TABLE 29.1** Examples of the Application of Electronic Noses and Tongues for Determination of Aroma and Taste in Wine Samples

| Application | Wine Types | Method/Tecnique | References |
|---|---|---|---|
| Analysis of the wine spoilage | Red and white Spanish wine | Potentiometric e-tongue and humid e-nose | Gil-Sánchez et al. (2011) |
| Quality evaluation | Chinese rice wines | E-eye (colorimeter), e-tongue (liquid cross-selective sensors), and e-nose (metal-oxide gas sensors) | Ouyang et al. (2014) |
| Fermentation monitoring | Italian wine | FT-NIR, FT-IR spectrometers, e-tongue (potentiometric array sensors), and e-nose (array of metal oxide semiconductor) | Buratti et al. (2011) |
| Organoleptic characteristics | Red wines vinified using different extraction techniques and micro-oxygenation methods and bottled using different closures | E-eye (UV–Vis Spectrophotometer), e-tongue (voltammetric carbon paste electrodes), and e-nose (array MOX) | Apetrei et al. (2012) |
| Discrimination of wines | Red wines prepared from *Vitis vinifera* (var. Grenache) | E-nose (MOX) and e-tongue (voltammetric carbon paste electrodes) | Rodriguez-Mendez et al. (2014) |
| Red wine aging monitoring | Spanish red wine | E-nose (based on resistive MOX sensors), e-tongue (based on voltammetric sensors), and e-eye (based on CIE Lab coordinates) | Prieto et al. (2011) |
| Differentiate and classify Barbera DOC wines | North italian DOC wine | Portable e-nose (PEN2—metal oxide semiconductor) and amperometric e-tongue (flow injection analysis) | Buratti et al. (2004) |
| Descriptors of Italian red dry wines of different DOC | Italian dry red wines of different denominations and vineyards | Portable e-nose (PEN2) and e-tongue (flow injection analysis with two amperometric detectors) | Buratti et al. (2007) |
| Wine analysis | Red wines | E-nose (porphyrin-based quartz microbalances sensor array) and e-tongue (potentiometric sensors) | Di Natale et al. (2004) |

of the Lombardia region (north of Italy). In this case, the potentiometric electrodes of the e-tongue were functionalized with thin films of metalloporphyrins deposited by electropolymerization technique. The e-nose and e-tongue data were compared with those obtained from chemical and sensorial analysis, to study the potential correlations among the different analytical approaches. The study showed that the combination of the e-nose and e-tongue offered better performances for the wine analysis and that the artificial sensing system was able to mimic the evaluation of a panel of trained human testers of several wine descriptors (Fig. 29.2).

A different combined system, constituted by an amperometric e-tongue and a commercial e-nose, was exploited for the analysis of four types of Barbera wines, produced in the northern region of Italy, with the aim to characterize and recognize the wines according to their denominations of origin (Buratti et al., 2004). In this case, the data of the combined e-nose and e-tongue were compared with those of chemical analysis and color evaluation.

In this work the commercial e-nose was a portable device (PEN2), composed of 10 metal oxide (MOX) semiconductor chemical sensors, equipped with a preconcentrator and a thermal desorber system. The e-tongue was constituted of two amperometric detectors coupled to a flow injection analysis system, with the wine samples delivered by dilution in a methanol/acetate buffer (70:30) carrier solution. The system was able to correctly differentiate Barbera wines according to their DOC (the Italian controlled denomination of origin) characteristic, with a small cross-validation error rate.

The same artificial sensorial system was later applied, together with spectrophotometric methods, to predict sensorial descriptors of Italian red dry wines of different DOC (Buratti et al., 2007), demonstrating a good accuracy in the prediction of most of the sensorial parameters evaluated.

More recently, the same group has reported the application of a panel composed of an e-tongue, an e-nose, FT-NIR, and FT-IR spectrometers to monitor the microfermentation of wine (Buratti et al., 2011). While the e-nose was the same of the previous studies, the e-tongue in this work was the taste-sensing system commercial device, based on potentiometric sensors. In this work, the spectroscopic technique was used to study molecular changes, while the e-nose and e-tongue evaluated the evolution of the aroma and taste profile during the must-wine fermentation.

A combined system, composed of a potentiometric e-tongue and a humid e-nose, has also been applied for the analysis of the deterioration of wine in contact with air (Gil-Sánchez et al., 2011). The potentiometric e-tongue was built with thick-film serigraphic techniques, using commercially available resistances and conductors for hybrid electronic circuits, that is, Ag, Au, Cu, Ru, AgCl, and C. The humid e-nose had an innovative design to respect the traditional

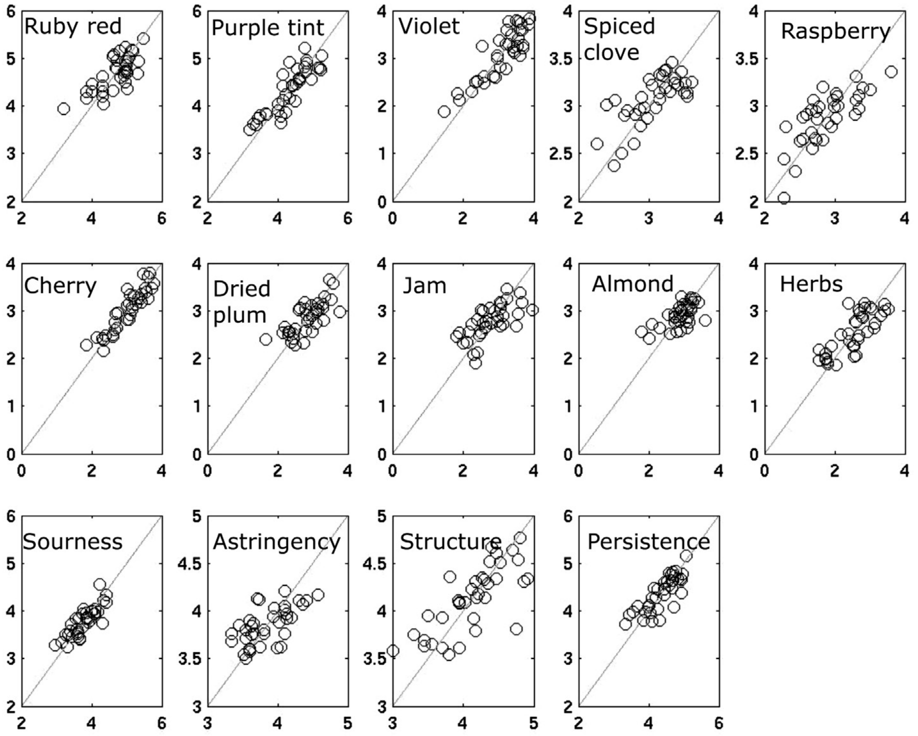

**FIGURE 29.2 Scatter plot of the 14 sensorial descriptors estimated by a PLS model built on the nose and tongue data set.** *(Reprinted with permission from Di Natale et al., 2004.)*

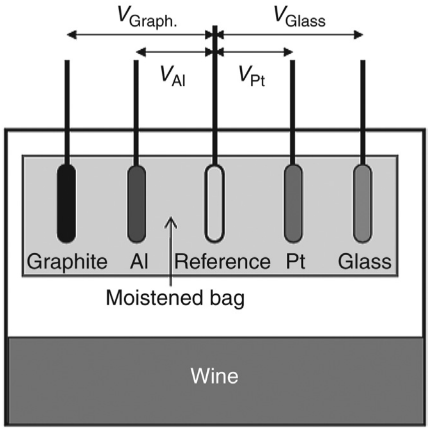

**FIGURE 29.3 Set-up of the humid e-nose.** *(Reprinted with permission from Gil-Sánchez et al., 2011.)*

arrays and wanted to emulate the working mechanism of the natural olfaction system, mimicking the wet environment of the nasal mucosa. This humid e-nose was composed of four electrodes, such as a pH glass electrode; aluminum, platinum, and graphite wires; and an Ag–AgCl reference electrode. The e-nose detected the volatiles originated by the wine samples and then dissolved in a moistened cloth bag (Fig. 29.3).

The e-tongue and the humid e-nose were exploited to monitor the spoilage of three Spanish wines (two red wines and one white wine) in contact with air after opening their bottles. The measurements were made up to 48 days after the bottle opening. The total acidity of the wines were contemporaneously made as a control of the sample spoilage. While the data analysis of the individual arrays showed a reduced discriminatory ability of the temporal evolution of the spoiled wines, indicating that the sample discrimination was not simply correlated with the increase of the wine acidity, the combined device demonstrated the ability to monitor the evolution of wine samples in the course of time (Fig. 29.4).

## 29.3 THE HYBRID E-TONGUE

In a slightly different approach, a hybrid system, where an e-tongue was combined with a optofluidic colorimetric system, has been proposed for wine analysis (Gutiérrez et al., 2010, 2011a). Different from the previous examples,

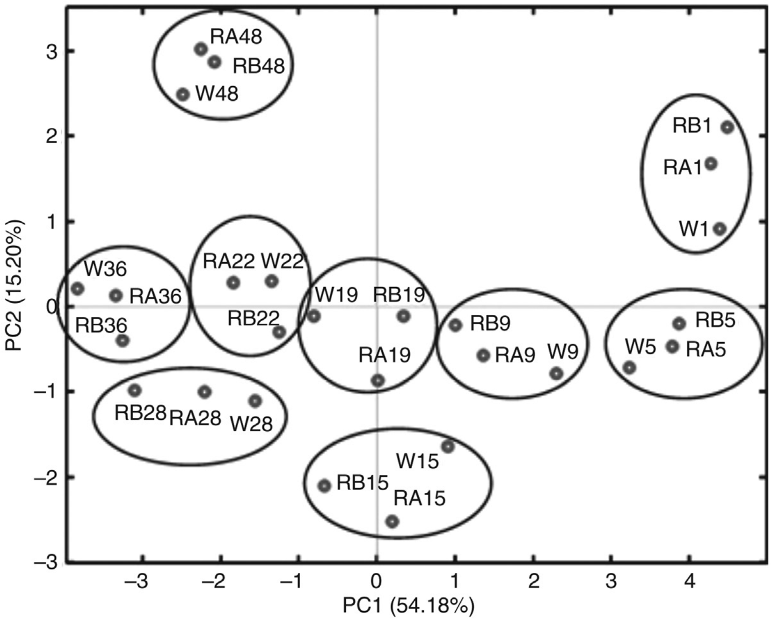

**FIGURE 29.4 PCA score plot obtained by combination of measurements of the e-tongue and the humid e-nose on two red wines *(RA, RB)* and a white wine *(W)*.** *(Reprinted with permission from Gil-Sánchez et al., 2011.)*

where both headspace (e-nose) and liquid phase (e-tongue) of the wine matrices contributed to the final result, in this case only the liquid phase was analyzed, joining an electrochemical and a colorimetric analysis of the wine samples, and for this reason the resulting device has been named a hybrid e-tongue. The system was composed of an array of four different kind of electrochemical sensors: six ion-sensitive field effect transistors (ISFETs), a conductivity sensor, a redox potential sensor, and two amperometric electrodes. The colorimetric analysis was performed by optofluidic system using fiber optics connected to a spectrometer. This hybrid e-tongue system was first tested for the characterization of red wines (Gutiérrez et al., 2010) and then for the quantification of the percentage of grape varieties in both red and white wines (Gutiérrez et al., 2011a,b). The results obtained showed that the hybrid e-tongue was effectively able to discriminate both red and white wine according to their grape variety composition and year of vintage, demonstrating a potential usefulness for the variety analysis in wine industry.

## 29.4 THE ELECTRONIC PANEL

A more complete system, called an "electronic panel" for wine assessment, has also been reported by Rodríguez-Méndez et al. (2004, 2014), Prieto et al. (2011), and Apetrei et al. (2012). The combined system is formed by an e-nose, based on array of commercial MOX sensors, a voltammetric e-tongue, based on the carbon paste electrodes (CPEs) modified with bisphthalocyanines, semiconducting polymers and perylenes, and an e-eye, which collected the transmittance spectra, recorded using 11 LEDs (covering the 780–380 nm spectral region) and calculating the CIELab coordinates. This electronic panel has been exploited for different scopes in wine analysis. In the first work (Rodríguez-Méndez et al., 2004), the combined system was applied to discriminate six red Spanish wines prepared using the same variety of grape (Tempranillo), but differing in their geographic origins and aging stages. The data analysis showed that the three independent devices offered uncorrelated information on the analyzed samples and their integration strongly increased the discrimination capability of the overall system, making the electronic panel suitable for the wine characterization.

The electronic panel was used to evaluate the influence of molecular oxygen interaction with wine samples on their organoleptic characteristics, both before (micro-oxygenation) and after bottling (nano-oxygenation) (Prieto et al., 2011). The experiments were performed on red wines having different polyphenols contents, depending on the vinification procedure adopted for their production. The influence of the different closures on the oxygenation transmission rates were also evaluated by the electronic panel. The solid-phase microextraction (SPME) technique was used to transfer the wine headspace into the e-nose chamber, with the aim to increase the concentration of the volatile organic compounds (VOC) presence in the wine flavor, and contemporaneously to reduce the ethanol and water content. Both ethanol and water, in fact, are not useful for the wine discrimination, since they are always present in the analyzed samples, but they can overwhelm the detection of the other VOCs, due their high concentrations. No sample treatment was necessary for both e-tongue and e-eye measurements. The three devices gave noncorrelated information on the analyzed samples, with the e-nose and e-eye more related to the closure influence and the e-tongue to the antioxidant character of the wine. As a result, the electronic panel showed a significant improvement of the discrimination performances, taking advantages of the overall independent information furnished by the individual components.

The same system was later exploited to monitor the red wine aging, with the aim to distinguish among the traditional wine aging process in oak barrels to respect the addition of oak chips in wines stored in stainless-steel tanks (Apetrei et al., 2012). The system was able to follow the change of the overall chemical composition of the wine samples, according to their different maturation environment, so allowing the discrimination of wines with the same origin, but different aging processes. The wine samples were also analyzed by the standard chemical analysis, to evaluate the chemical composition changes due to the aging process. The results obtained showed the possibility to use the electronic panel to predict the method exploited for the wine maturation; although the same discrimination could be possible by chemical analysis, it is worth noting that the electronic panel, giving a global assess of the target sample, can offer several advantages, such as, for example, the rapidity of the analysis, minimal pretreatment of the sample, single measure, and so on.

More recently the electronic panel, this time composed by the e-nose and e-tongue, was applied for the evaluation oxygen exposure levels and polyphenolic content of red wines (Rodríguez-Méndez et al., 2014). Twenty-five chemical parameters of the analyzed wines were obtained by traditional chemical analysis and related to the chemical composition of the wines, such as the polyphenols' content, pigments, and oxygen amount. The results obtained showed a good correlation between the responses obtained by the electronic panel and the chemical parameters measured by chemical analysis, demonstrating that the combined device could offer also quantitative data related to chemical composition, other than a global assessment of the analyzed wines.

Following a similar approach, the exploitation of a hybrid system was also tested for the quality assessment of Chinese rice wine samples (Ouyang et al., 2014). The system was composed by three commercial devices, an e-eye (ColorQuest XE colorimeter), an e-nose (Airsense Analytics PEN3), and an e-tongue (αAstree Alpha M.O.S), with the aim to mimic the integration of the human senses in the global evaluation of wine. Seventy-five samples of a Chinese rice wine brand were analyzed with the combined system, without sample pretreatment. To explore the correlation of the electronic panel results with the human sense evaluation, the rice wine samples have been evaluated also by a trained human panel test, which assigned the score sensory attributes to the analyzed samples in three variables crossed perception: color, aroma, and taste. In this work, particular attention was devoted to the data analysis and the results obtained demonstrated that the multisensor's data fusion developed efficiently to mimic the performances of human tests, making the system promising for the exploitation in the real field.

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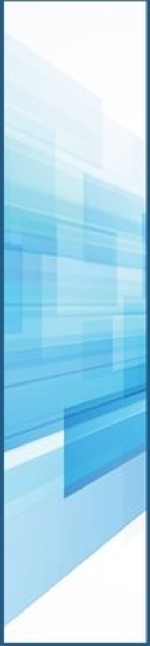

# ELECTRONIC NOSES AND TONGUES IN FOOD SCIENCE

## Edited by María Luz Rodríguez Méndez

*Electronic Noses and Tongues in Food Science* examines the latest technologies of these instruments which are the result of the combination of arrays of sensors, microprocessors, advanced informatics, and statistics. They include resistive, optical, electrochemical, or piezoelectric platforms, where a variety of sensing materials can be immobilized using numerous different techniques. This book provides examples of the use of electronic noses and tongues to characterize foods and beverages from ripening to harvesting, and from storage of raw materials to packaging and consumption.

Divided into three parts, the book opens with an examination of the electronic nose. Chapters within this part cover the principles and applications of the electronic nose, including, among others, dairy products, fish, fruit ripeness, and meat contamination. Part two moves on to analyze the electronic tongue. In this part, chapters review topics such as wine and beer analysis, meat and fish spoilage, and drinking water analysis, for example. The final part of the volume explores the combined electronic nose and tongue, and its use in wine, alcoholic fermentation, and olive oil.

A key reference for food scientists, technologists, food industry workers, and researchers, *Electronic Noses and Tongues in Food Science* provides a thorough overview of the technology and its applications in a variety of foodstuffs.

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